

This Page Is Inserted by IFW Operations  
and is not a part of the Official Record

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representation of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

## **IMAGES ARE BEST AVAILABLE COPY**

**As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.**



US 20020160139A1

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2002/0160139 A1  
Huang et al. (43) Pub. Date: Oct. 31, 2002

(54) POLYMER SURFACE MODIFICATION

(75) Inventors: Jiang Huang, San Jose, CA (US);  
Shoujun Xiao, Foster City, CA (US);  
Marc A. Unger, South San Francisco,  
CA (US)

Correspondence Address:  
TOWNSEND AND TOWNSEND AND CREW,  
LLP  
TWO EMBARCADERO CENTER  
EIGHTH FLOOR  
SAN FRANCISCO, CA 94111-3834 (US)

(73) Assignee: Fluidigm Corporation, South San Francisco, CA (US)

(21) Appl. No.: 10/118,467

(22) Filed: Apr. 5, 2002

Related U.S. Application Data

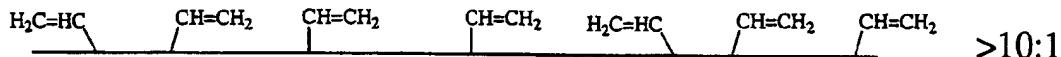
(60) Provisional application No. 60/281,929, filed on Apr. 6, 2001.

Publication Classification

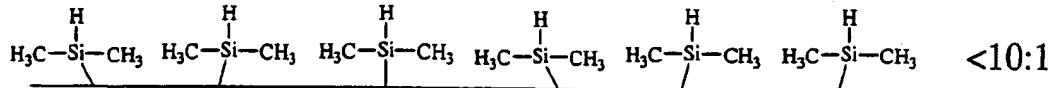
(51) Int. Cl.<sup>7</sup> B32B 1/06; B05D 7/22; B05D 3/10  
(52) U.S. Cl. 428/36.9; 427/337; 427/230;  
428/451; 428/447; 428/36.91

(57) ABSTRACT

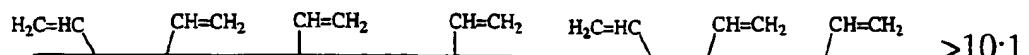
The present invention is directed to a surface modified polymer comprising a surface which is covalently bonded to a surface modifying compound. Formation of the covalent bond between the polymer and the surface modifying compound is achieved by a reaction between an intrinsic functional group that is present in the polymer and the functional group of the surface modifying compound. By using a polymer having an intrinsic functional group, a separate surface activation step is avoided.



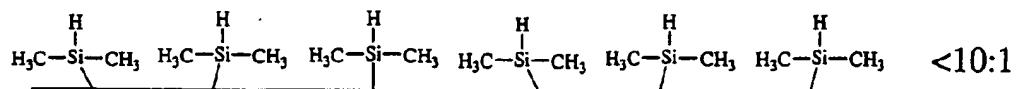
Vinyl excess silicone (only functional groups on the surface are shown)



Silane excess silicone (only functional groups on the surface are shown)

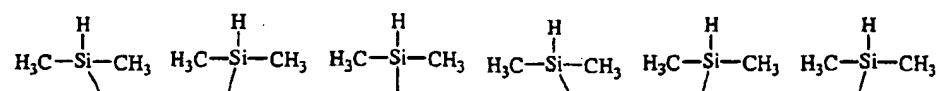


Vinyl excess silicone (only functional groups on the surface are shown)



Silane excess silicone (only functional groups on the surface are shown)

Figure 1A



Silane excess silicone (only functional groups on the surface are shown)

PEG,  
Pt Catalyst

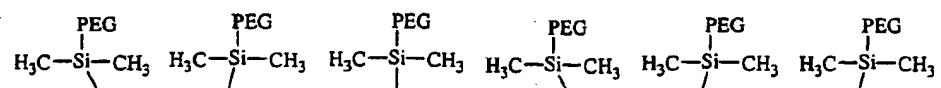
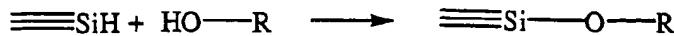


Figure 1B. Surface modification reaction of a silane functional group and PEG.

Silicone surface modification route I:



Where R = -C<sub>18</sub>, -C<sub>8</sub>, -C<sub>4</sub>, -phenyl, -PEG, -(CH<sub>2</sub>)<sub>x</sub>-(CF<sub>2</sub>)<sub>y</sub>-CF<sub>3</sub>, etc.

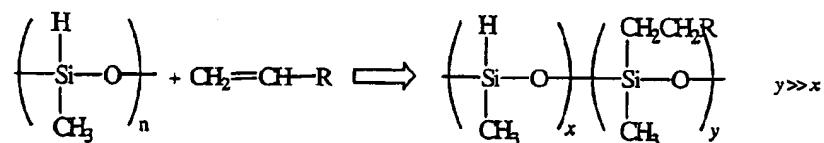
Silicone surface modification route II:



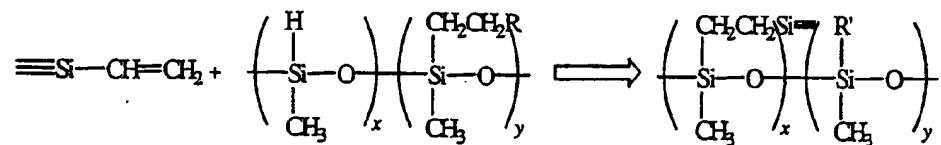
Where R' = -C<sub>18</sub>, -C<sub>8</sub>, -C<sub>4</sub>, -phenyl, -PEG, -(CH<sub>2</sub>)<sub>x</sub>-(CF<sub>2</sub>)<sub>y</sub>-CF<sub>3</sub>, etc.

Silicone surface modification route III:

Step 1.



Step 2.



Where R' = -C<sub>18</sub>, -C<sub>8</sub>, -C<sub>4</sub>, -phenyl, -CH<sub>2</sub>CH<sub>2</sub>-PEG, -(CH<sub>2</sub>)<sub>x</sub>-(CF<sub>2</sub>)<sub>y</sub>-CF<sub>3</sub>, etc.

Figure 2

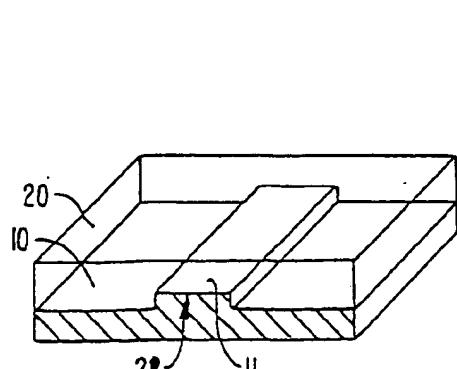


FIG. 3

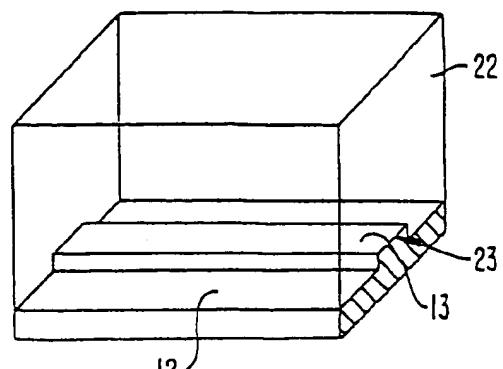


FIG. 4

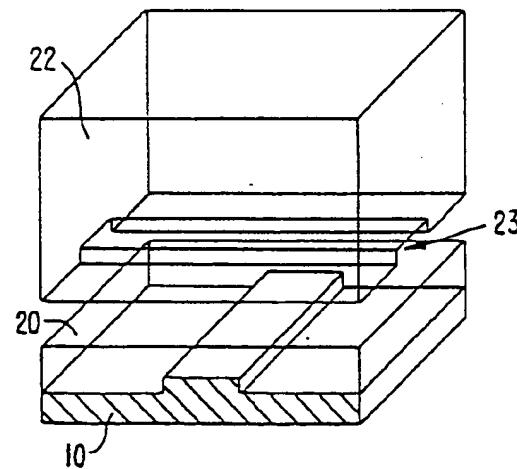


FIG. 5

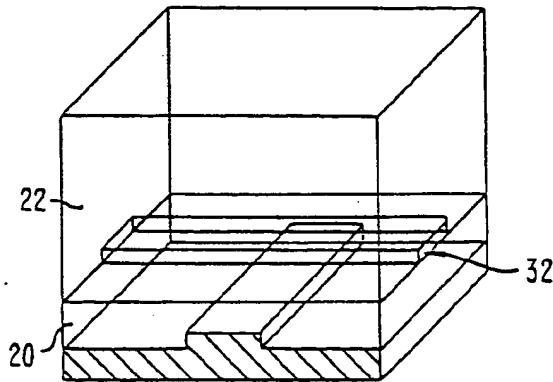
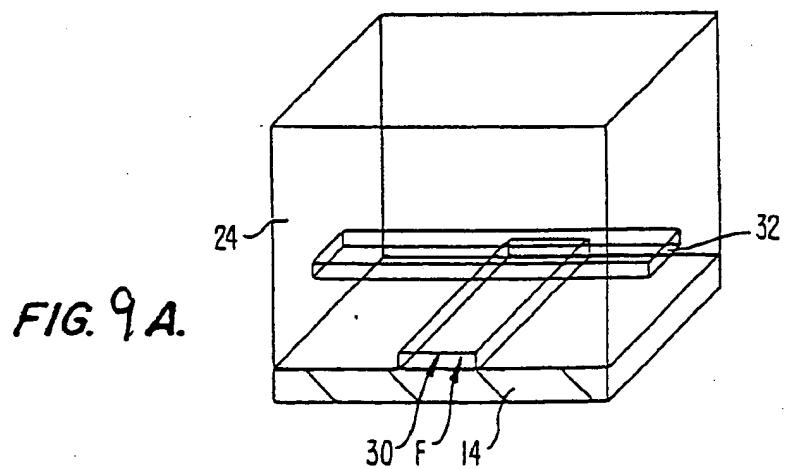
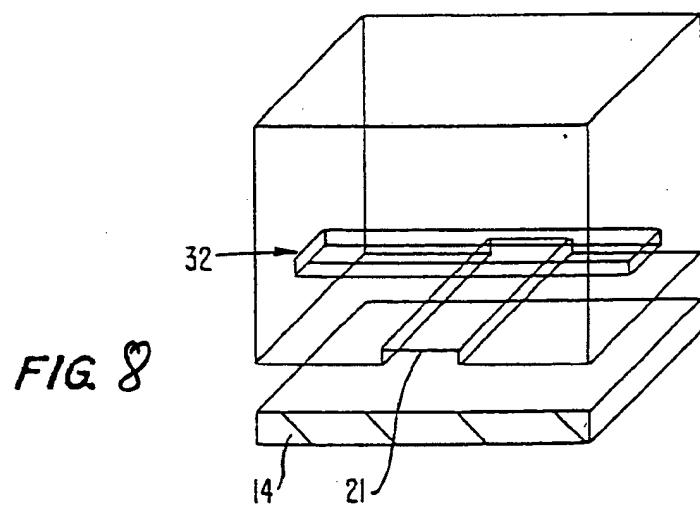
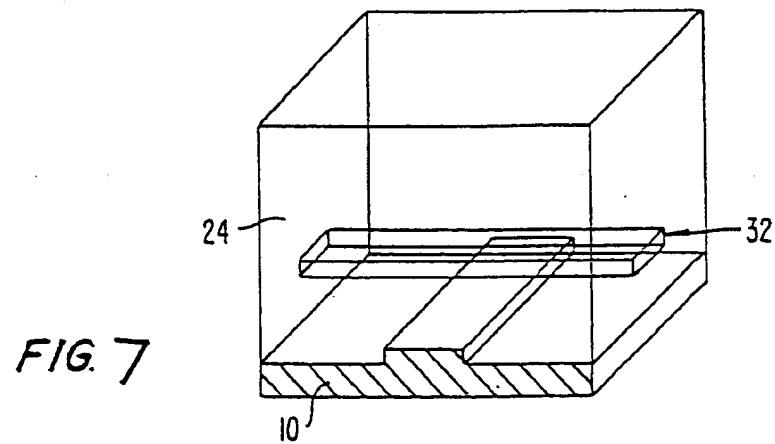


FIG. 6



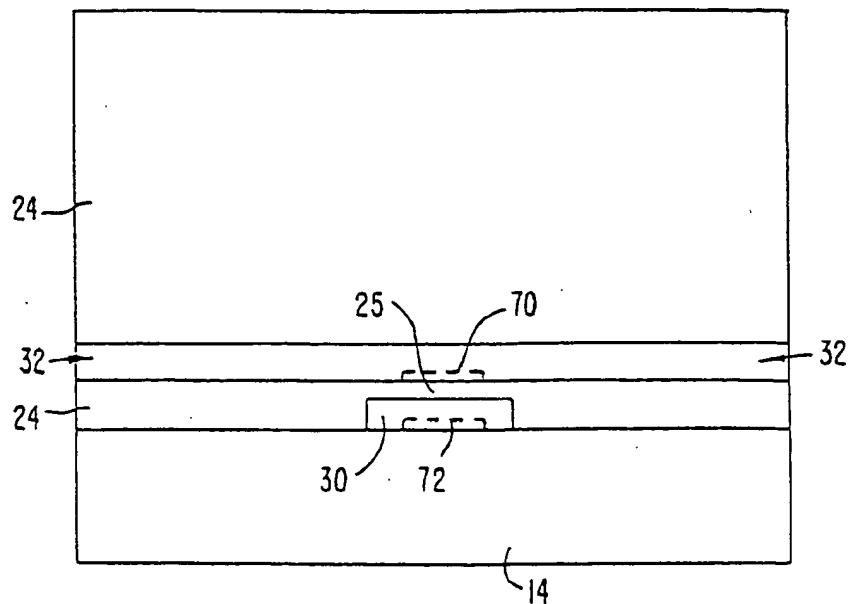


FIG. 9B.

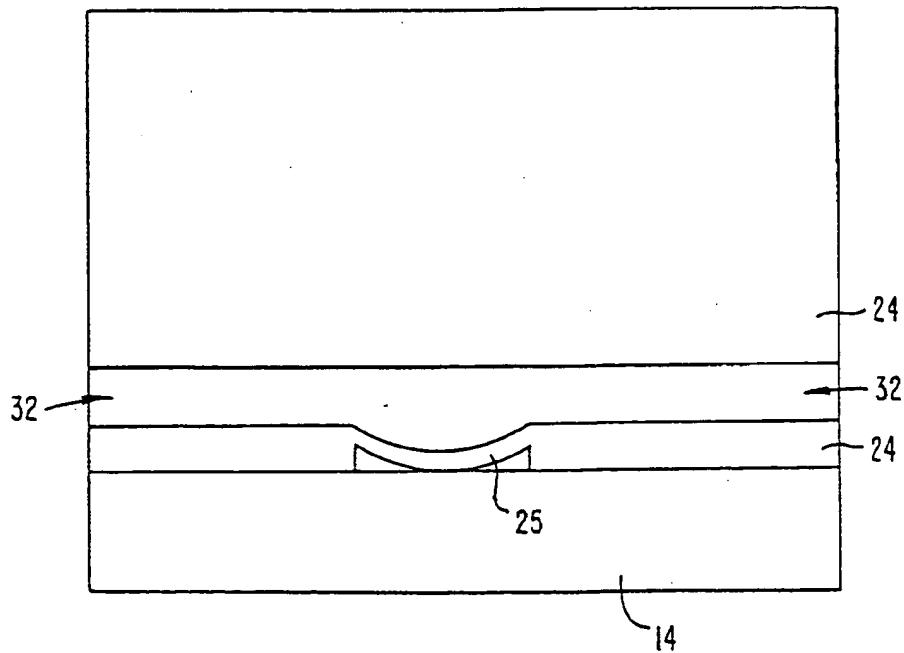


FIG. 9C

FIG. 10

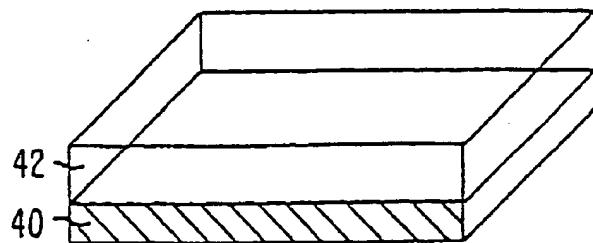


FIG. 11

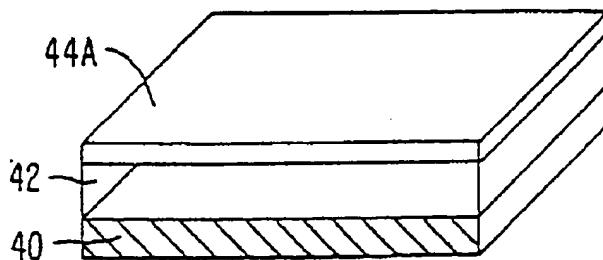


FIG. 12

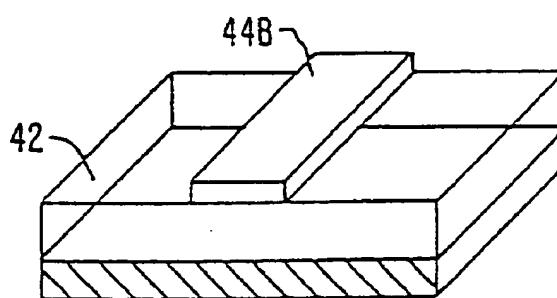
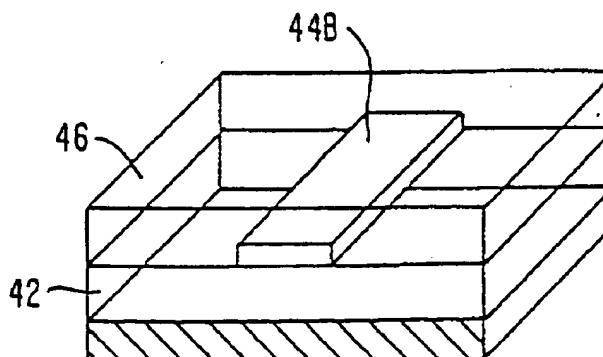


FIG. 13



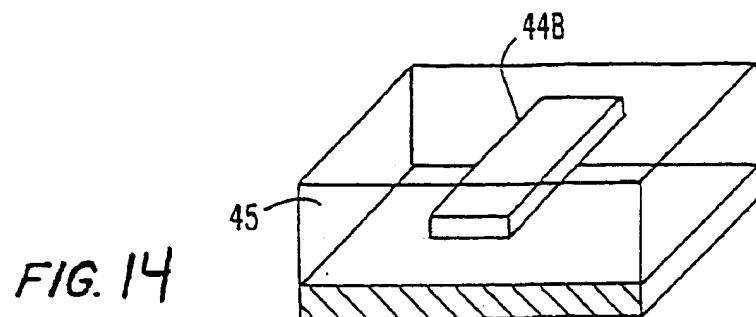


FIG. 14

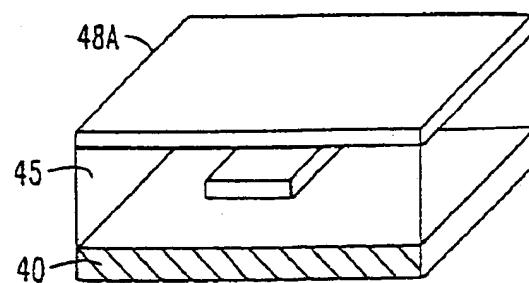


FIG. 15

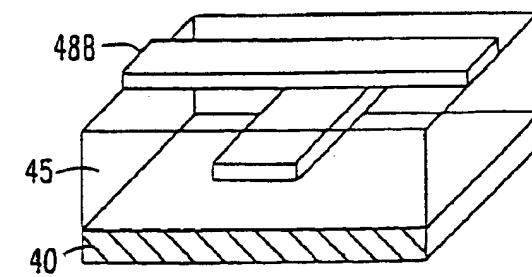


FIG. 16

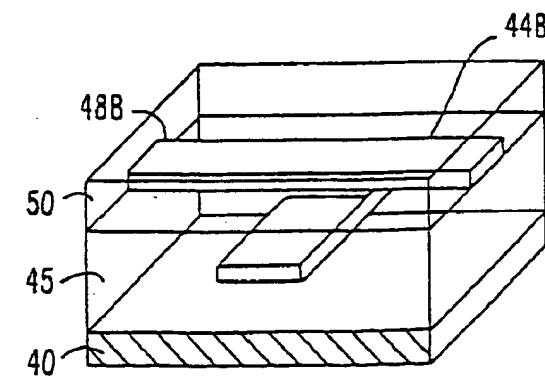


FIG. 17

FIG. 18

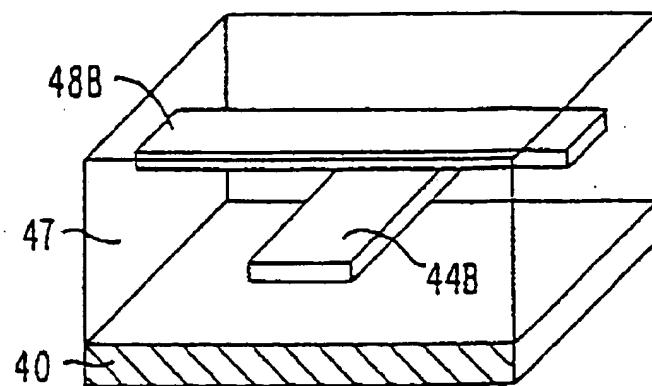


FIG. 19

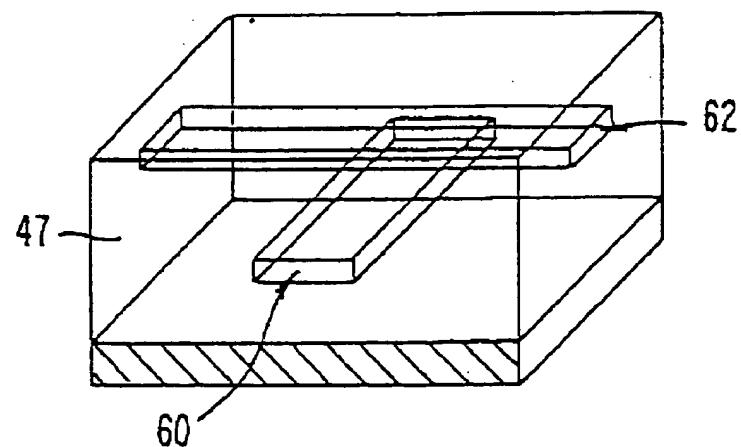
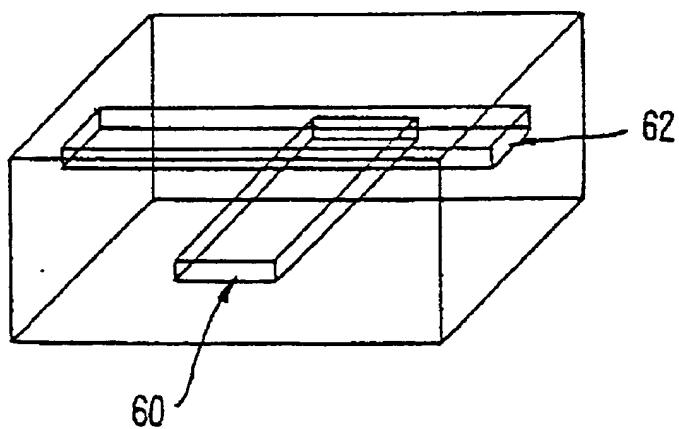
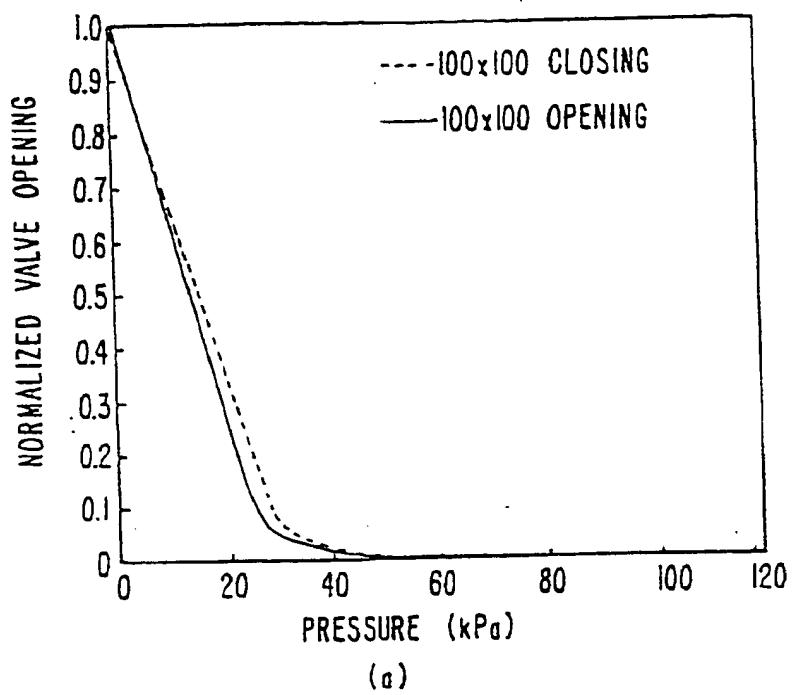
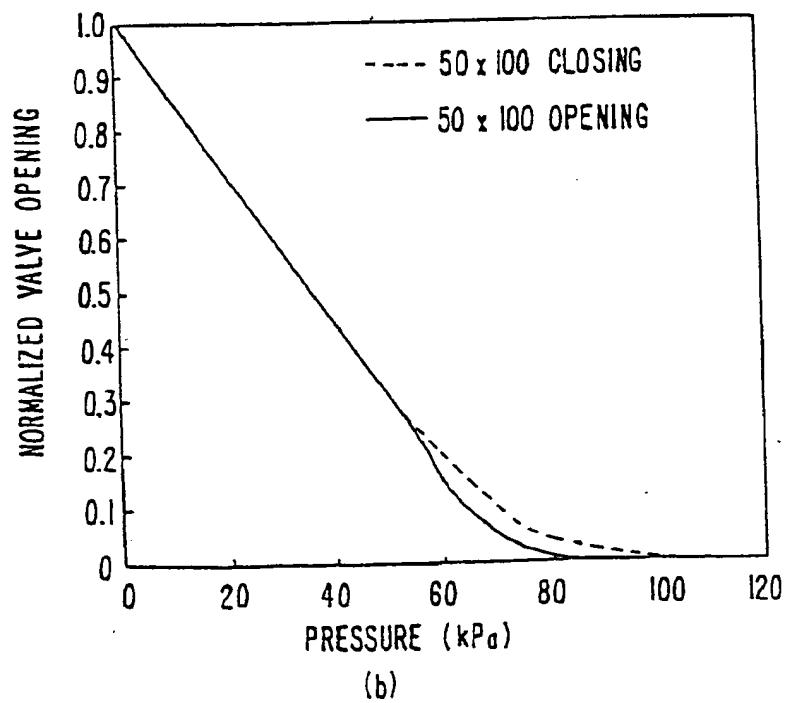


FIG. 20





(a)



(b)

FIG. 21

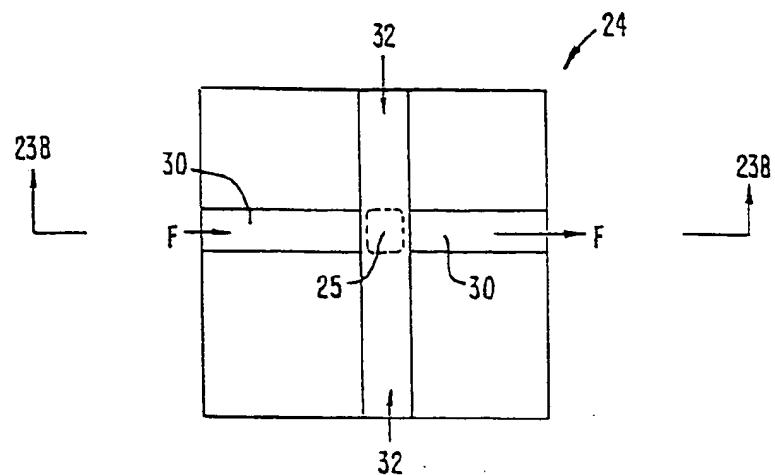


FIG. 22A.

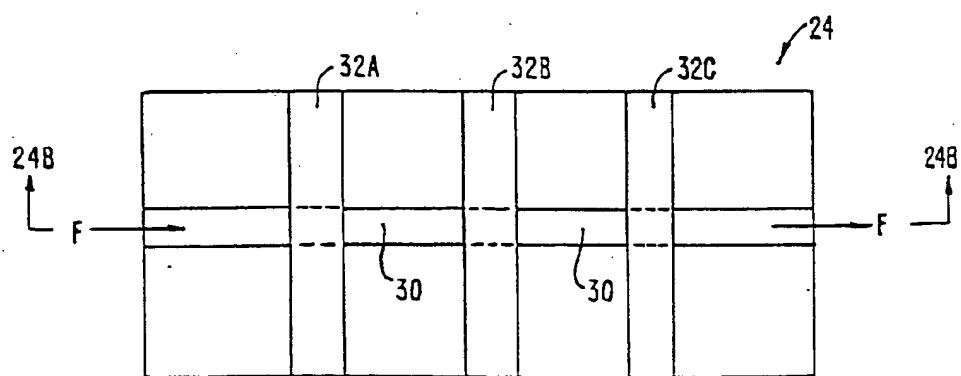


FIG. 23A.

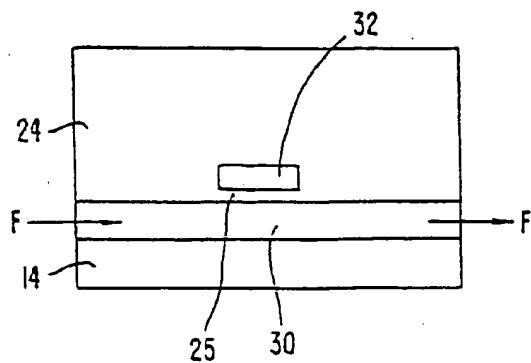


FIG. 22.B.

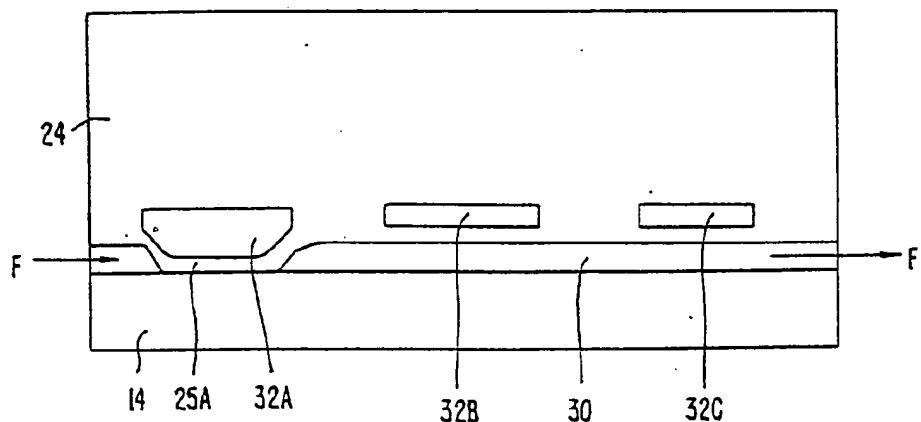


FIG. 23.B.

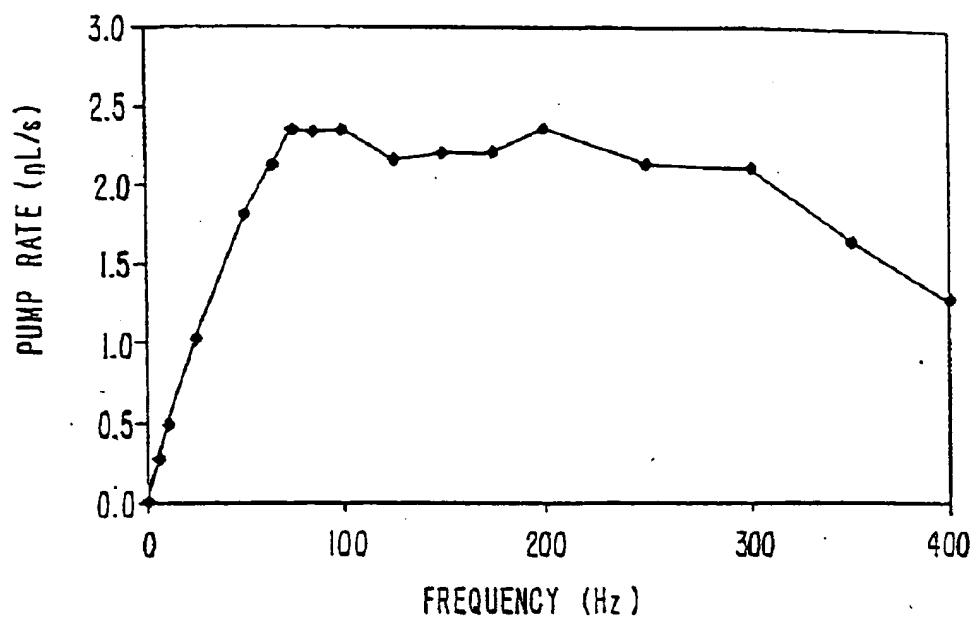


FIG. 24

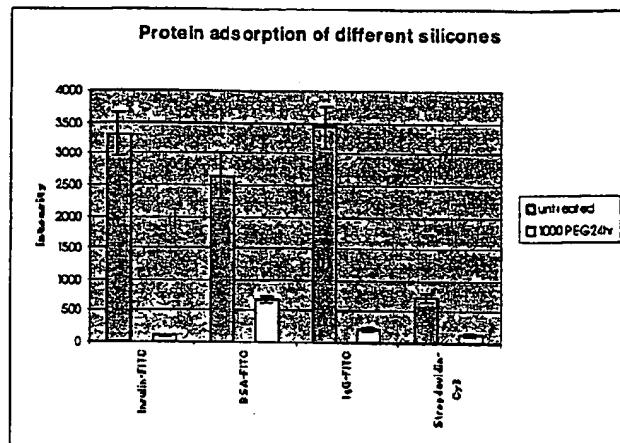


Figure 25

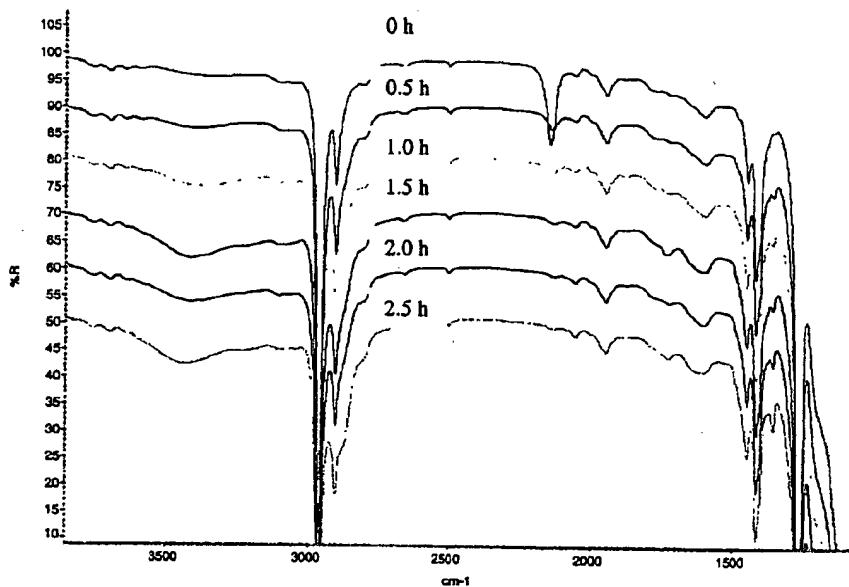


Figure 26

ATR-FTIR spectra showing surface grafting of AG-160 to GE RTV 615 4:1 PDMS. PDMS (4:1) pieces ( $1.0 \times 1.5 \times 4.0$  cm) were immersed in 5 g pure AG-160 with 10 mg  $H_2PtCl_6$  to carry the reaction on at  $80^\circ C$  for different times shown.

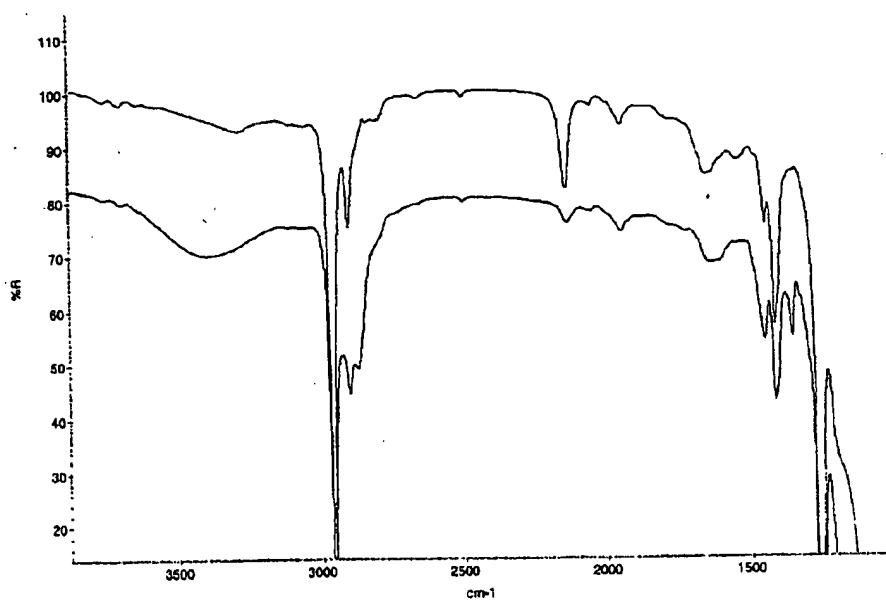


Figure 27

ATR-FTIR spectra of fibrinogen adsorption behavior of the PDMS (4:1) and AG-160-grafted PDMS surfaces.

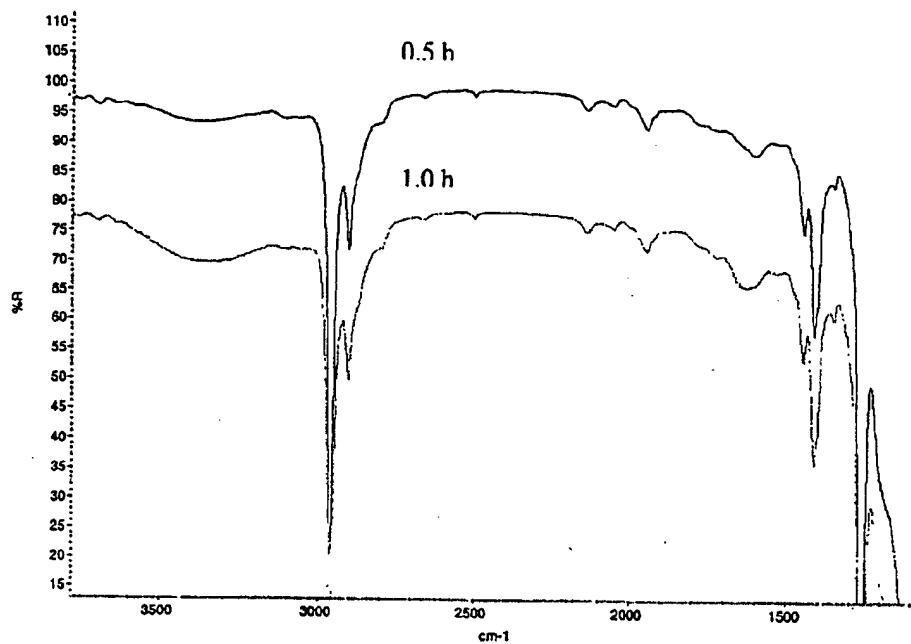


Figure 28

ATR-FTIR spectra showing surface grafting of AG-160 in water solution to GE 4:1 PDMS. PDMS (4:1) samples ( $1.0 \times 1.5 \times 4.0$  cm) were immersed in 25 ml of AG-160 (10 wt%) aqueous solution with 4 mg  $H_2PtCl_6$  catalyst at  $80^\circ C$  for different times shown.

**POLYMER SURFACE MODIFICATION****CROSS-REFERENCES TO RELATED APPLICATIONS**

[0001] This application claims the benefit of U.S. Provisional Application No. 60/281,929, filed Apr. 6, 2001, which is incorporated herein by reference in its entirety.

**FIELD OF THE INVENTION**

[0002] The present invention relates to a surface modified polymer comprising an exterior surface containing a covalently bonded surface modifying compound and a bulk matrix comprising a reactive polymerizable functional group dispersed therein, and a method for using the same.

**BACKGROUND OF THE INVENTION**

[0003] Polymers are useful in a variety of applications, including fundamental research and biomedical applications such as solid phase diagnostics, drug delivery, biomaterials, and other areas. Unfortunately, many polymer materials are incompatible with a variety of reagents in many applications. For example, proteins and other biomolecules tend to adhere to hydrophobic surfaces, and therefore hydrophobic polymers may not be suitable in some applications where proteins and other biomolecules are involved. Or some type of surface modification/coating will be needed to reduce the non-specific binding (NSB) of these biomolecules to hydrophobic polymer surfaces.

[0004] A recent success of fabricating microfluidic devices using an elastic polymer has further increased the utility of polymers in fundamental research and biological applications. Successful fabrication of microfluidic devices from elastic polymers has been particularly useful in small scale sample preparation, chemical synthesis, drug delivery, biomedical devices, and other applications where a micro-scale device is required. Because of a much higher surface area to volume ratio in microfluidic environment, the adsorption of reagents on the surface of the microfluidic devices result in significant changes in reagent concentration and carryover contamination, etc. Therefore, reducing NSB of molecules onto the microfluidic device surface is particularly important.

[0005] Recently, surface modification or coating has become a more and more popular way to create "new" materials by selecting core polymer materials for their mechanical properties and then modifying or coating the polymer surface to fit a particular environment. The desired surface properties can be biocompatibility, chemical resistance, wettability, non-stick, etc. Preferably, a surface modification process should be simple, form a covalent bond, and inexpensive. Moreover, it should be mild so as not to degrade the substrate polymer. Commonly used surface modification/coating techniques include plasma deposition, physical vapor deposition, chemical vapor deposition, ion bombardment, ion-beam sputter deposition, ion-beam-assisted deposition, sputtering, thermal spraying, and dipping. Each method has its advantages and disadvantages. For example, if the coating material is not permanently bonded to the polymer, it needs to be reapplied frequently to maintain inertness of the polymer to the reagent.

[0006] Inability to produce a permanent coating is particularly true for polymers with low surface energy, such as,

polyethylene, poly(dimethylsiloxane) (PDMS or silicone polymer), poly(tetrafluoroethylene) (PTFE, Teflon®). In addition, microfluidic devices have extremely small features, e.g., flow channels having width of as small as 1  $\mu\text{m}$ . Therefore, coating a surface modifying compound may destroy the microfluidic design or render the microfluidic device unusable.

[0007] Conventional permanent bonding (grafting) of a surface modifying compound to an organic or inorganic substrate (i.e., glass, silicon, metal, polymer, etc.) requires activation of the substrate surface, i.e., introducing a reactive functional group on the substrate surface. Typically, the substrate surface is activated by ion deposition. Other methods of surface activation include treating with an oxidizing agent, a reducing agent, or a plasma, such as oxygen plasma or water plasma. After a reactive functional group is introduced on the substrate surface, it is reacted with a surface modifying compound to form a covalent bond. Alternatively, the activated surface is reacted with a linker compound which serves as a linker between the substrate surface and the surface modifying compound. Unfortunately, many linkers and solvents used in these processes are not compatible with a large amount of polymeric materials. Also, surface activation processes increase the cost and time for producing such polymeric devices. Moreover, many polymer surface activation processes result in activation of only a small portion of the polymer surface.

[0008] Therefore, there is a need for a process of producing a surface modified polymer that does not require a separate polymer surface activation process. There is also a need for microfluidic devices comprising a covalently attached surface modifying compound.

**SUMMARY OF THE INVENTION**

[0009] One aspect of the present invention provides a surface modified polymer comprising:

[0010] a bulk polymer matrix comprising an intrinsic functional group dispersed therein; and

[0011] a polymeric surface which comprise a surface modifying compound that is covalently bonded to the intrinsic functional group.

[0012] Preferably, the bulk matrix comprises at least 1 reactive functional group per 10,000 monomeric units of said polymer.

[0013] In one embodiment, the surface modified polymer comprises a channel extending from an exterior surface of the polymer through the bulk polymer matrix, wherein the channel defines an inner surface. Preferably, the inner surface comprises a surface modifying compound that is covalently bonded to the reactive functional group.

[0014] In another embodiment, the surface modified polymer comprises a plurality of layers of said bulk polymer matrix, wherein each layer of said bulk polymer matrix comprises a complementary reactive functional group dispersed therein. In this particular embodiment, the interface between two bulk polymer matrix layers is, preferably, covalently bonded through polymerization of the complementary reactive functional groups of said bulk polymer matrix layer.

[0015] In one particular embodiment, the surface modified polymer is produced from an off-ratio polymer. Preferably, the surface modified polymer is produced from a silicone off-ratio polymer. More preferably, the silicone off-ratio polymer is room temperature vulcanizing silicone (e.g., RTV).

[0016] In another embodiment, the surface modified polymer comprises at least two layers of GE RTV 615 polymers in which the reactive functional groups of two adjoining layers are such that the reactive functional group of one layer is silane and the reactive functional group of the other layer is an olefin.

[0017] Still in another embodiment, the intrinsic functional group is selected from the group consisting of silane, olefin, isocyanate, hydroxyl, epoxy, and amine.

[0018] Yet in another embodiment, the surface modified polymer is derived from polymerization of at least two different monomeric components. Preferably, the reactive functional groups of a first and second monomeric components are complementary reactive functional groups of each other selected from the group consisting of olefin, silane, hydroxyl, isocyanate, epoxy, amine and a combination thereof.

[0019] In one embodiment, the surface modifying compound is a biocompatible compound, thereby rendering the surface modified polymer biocompatible. Preferably, the biocompatible polymer is selected from the group consisting of vinyl terminated small molecules, acrylate terminated small molecules, polyethylene glycol, poly(methyl methacrylate), poly(ethylene vinyl acetate), poly(2-hydroxyethyl methacrylate), polyvinylpyrrolidone,  $\text{CH}_2=\text{CH-PEG}$  (i.e., allyl-PEG), and  $\text{CH}_2=\text{CH-(CH}_2\text{)}_n\text{-Teflon}$ .

[0020] Another aspect of the present invention provides a polymeric article comprising:

[0021] an exterior surface;

[0022] a bulk polymer matrix comprising an intrinsic functional group dispersed therein; and

[0023] a channel extending from the exterior surface through the bulk polymer matrix, wherein the channel defines an inner surface,

[0024] wherein the exterior and inner surfaces comprise a surface modifying compound that is covalently bonded to the intrinsic functional group.

[0025] In one embodiment, the polymeric article is a microfluidic device. Preferably, the channel defines a fluid flow channel of the microfluidic device.

[0026] In one particular embodiment, the surface modifying compound that is covalently attached to the inner surface of flow channel is a stationary phase compound which is capable of separating an analyte in a fluid sample.

[0027] In another embodiment, the surface modifying compound that is covalently attached to said exterior surface is a biocompatible compound.

[0028] Another aspect of the present invention provides a method for modifying a physical property of a polymer surface comprising:

[0029] (a) forming a bulk polymer matrix having an intrinsic functional group dispersed therein; and

[0030] (b) producing a surface modified polymer by contacting a surface of the polymer with a surface modifying compound comprising a complementary functional group under conditions sufficient to form a covalent bond between the intrinsic functional group that is present on the polymer surface and the complementary functional group of the surface modifying compound.

[0031] In one embodiment, the intrinsic functional group of the bulk polymeric matrix is selected from the group consisting of silane, olefin, isocyanate, hydroxyl, epoxy, and amine.

[0032] In one particular embodiment, the surface modifying compound render the polymer resistant to a solvent. Preferably, the surface modifying compound is selected from the group consisting of  $\text{CH}_2=\text{CH-(CH}_2\text{)}_n\text{-Teflon}$ ,  $\text{CH}_2=\text{CH-(CH}_2\text{)}_n\text{-fluoropolymer}$ , a fluoropolymer containing an acrylate functional group,  $\text{CH}_2=\text{CH-(CH}_2\text{)}_n\text{-CH}_3$ , where  $n$  is an integer from 0 to 30, and preferably from 0 to 20.

[0033] In another embodiment, the bulk polymer matrix forming step comprises admixing a first monomeric compound comprising a first reactive functional group with a second monomeric compound comprising a second reactive functional group under conditions sufficient to produce the bulk polymer matrix, wherein the first reactive functional group and the second reactive functional group are complementary functional groups. Preferably, the first reactive functional group and the second reactive functional group are selected from the group consisting of silane, olefin, hydroxyl, isocyanate, epoxy, and amine, such that the first reactive functional group and the second reactive functional group are complementary functional groups.

[0034] Still another aspect of the present invention provides a microfluidic device comprising:

[0035] (a) a bulk polymer matrix comprising an intrinsic functional group dispersed therein;

[0036] (b) a flow channel within said bulk polymer matrix, wherein said flow channel defines an inner surface; and

[0037] (c) a surface modifying compound covalently attached to the intrinsic functional group that is present on the inner surface.

[0038] Preferably, the bulk polymer matrix comprises at least one reactive functional group per 1000 monomeric unit used to form said bulk polymer matrix.

[0039] In one embodiment, the microfluidic device comprises a plurality of layers of said bulk polymer matrix, wherein each layer of said bulk polymer matrix comprises a reactive functional group dispersed therein. Preferably, two adjoining bulk polymer matrix layers are covalently bonded to one another by the reactive functional groups that are complementary to one another.

[0040] In one particular embodiment, the intrinsic functional group is selected from the group consisting of silane, olefin, hydroxyl, isocyanate, epoxy, and amine.

[0041] Preferably, the bulk polymer matrix is derived from polymerization of at least two different monomeric components. In one particular embodiment, the intrinsic functional group of a first monomeric component and the intrinsic functional group of a second monomeric component are complementary to each other and are selected from the group consisting of olefin, silane, hydroxyl, isocyanate, epoxy, amine, and a combination thereof.

[0042] In one embodiment, the surface modifying compound is a biocompatible polymer. In this manner, the microfluidic device can be used in various biophysical applications, including as medical devices, assay devices, sample separation devices, etc. Preferably, the biocompatible polymer is selected from the group consisting of polyethylene glycol, poly(methyl methacrylate), poly(ethylene vinyl acetate), poly(2-hydroxyethyl methacrylate), polyvinylpyrrolidone,  $\text{CH}_2=\text{CH-PEG}$ , and  $\text{CH}_2=\text{CH-(CH}_2\text{)}_n\text{-Teflon}$ ®.

[0043] Yet another aspect of the present invention provides a method for producing a microfluidic device comprising a bulk polymer matrix, a flow channel within said bulk polymer matrix, wherein the flow channel defines an inner surface, and a surface modifying compound covalently attached to the inner surface, said method comprising the steps of:

[0044] (a) producing the microfluidic device from a polymer precursor under conditions sufficient to produce the bulk polymer matrix comprising an intrinsic functional group dispersed within the inner surface, and

[0045] (b) contacting the inner surface with a surface modifying compound under conditions sufficient to covalently attach the surface modifying compound to the inner surface.

[0046] Preferably, the bulk polymer matrix comprises at least one reactive functional group per 10,000 monomeric units of said polymer.

[0047] In one embodiment, the polymer precursor comprises at least two different monomeric components. Preferably, the reactive functional group of a first monomeric component and the reactive functional group of a second monomeric component are a complementary reactive functional group of each other selected from the group consisting of an olefin, silane, hydroxyl, isocyanate, epoxy, and amine.

#### BRIEF DESCRIPTION OF THE DRAWINGS

[0048] FIG. 1A is a schematic illustration of off-ratio silicone polymers having different intrinsic functional groups on its surface depending on the ratio of each component used.

[0049] FIG. 1B illustrates a surface modification reaction between a silane functional group and polyethylene glycol (i.e., PEG).

[0050] FIG. 2 illustrates a variety of methods for modifying a silicone polymer surface.

[0051] FIG. 3 is an illustration of a first elastomeric layer formed on top of a micromachined mold.

[0052] FIG. 4 is an illustration of a second elastomeric layer formed on top of a micromachined mold.

[0053] FIG. 5 is an illustration of the elastomeric layer of FIG. 4 removed from the micromachined mold and positioned over the top of the elastomeric layer of FIG. 3.

[0054] FIG. 6 is an illustration corresponding to FIG. 5, but showing the second elastomeric layer positioned on top of the first elastomeric layer.

[0055] FIG. 7 is an illustration corresponding to FIG. 6, but showing the first and second elastomeric layers bonded together.

[0056] FIG. 8 is an illustration corresponding to FIG. 7, but showing the first micromachined mold removed and a planar substrate positioned in its place.

[0057] FIG. 9A is an illustration corresponding to FIG. 8, but showing the elastomeric structure sealed onto the planar substrate.

[0058] FIGS. 9B is a front sectional view corresponding to FIG. 9A, showing an open flow channel.

[0059] FIG. 9C corresponds to FIG. 9A, but shows a first flow channel closed by pressurization in second flow channel.

[0060] FIG. 10 is an illustration of a first elastomeric layer deposited on a planar substrate.

[0061] FIG. 11 is an illustration showing a first sacrificial layer deposited on top of the first elastomeric layer of FIG. 10.

[0062] FIG. 12 is an illustration showing the system of FIG. 11, but with a portion of the first sacrificial layer removed, leaving only a first line of sacrificial layer.

[0063] FIG. 13 is an illustration showing a second elastomeric layer applied on top of the first elastomeric layer over the first line of sacrificial layer of FIG. 12, thereby encasing the sacrificial layer between the first and second elastomeric layers.

[0064] FIG. 14 corresponds to FIG. 13, but shows the integrated monolithic structure produced after the first and second elastomer layers have been bonded together.

[0065] FIG. 15 is an illustration showing a second sacrificial layer deposited on top of the integral elastomeric structure of FIG. 14.

[0066] FIG. 16 is an illustration showing the system of FIG. 15, but with a portion of the second sacrificial layer removed, leaving only a second line of sacrificial layer.

[0067] FIG. 17 is an illustration showing a third elastomer layer applied on top of the second elastomeric layer and over the second line of sacrificial layer of FIG. 16, thereby encapsulating the second line of sacrificial layer between the elastomeric structure of FIG. 14 and the third elastomeric layer.

[0068] FIG. 18 corresponds to FIG. 17, but shows the third elastomeric layer cured so as to be bonded to the monolithic structure composed of the previously bonded first and second elastomer layers.

[0069] FIG. 19 corresponds to FIG. 18, but shows the first and second lines of sacrificial layer removed so as to provide two perpendicular overlapping, but not intersecting, flow channels passing through the integrated elastomeric structure.

[0070] FIG. 20 is an illustration showing the system of FIG. 19, but with the planar substrate thereunder removed.

[0071] FIGS. 21a and 21b illustrate valve opening vs. applied pressure for various flow channel dimensions.

[0072] FIG. 22A is a top schematic view of an on/off valve.

[0073] FIG. 22B is a sectional elevation view along line 23B-23B in FIG. 22A

[0074] FIG. 23A is a top schematic view of a peristaltic pumping system.

[0075] FIG. 23B is a sectional elevation view along line 24B-24B in FIG. 23A

[0076] FIG. 24 is a graph showing experimentally achieved pumping rates vs. frequency for an embodiment of the peristaltic pumping system of FIGS. 23A and 23B.

[0077] FIG. 25 is a graph showing the PEG-bound surface modified polymer significantly reduces the adsorption of proteins.

[0078] FIG. 26 is an ATR-FTIR spectra of the surfaces of the 4:1 treated PDMS samples of Example 2 as a function of reaction time.

[0079] FIG. 27 is an ATR-FTIR spectra of protein (fibrinogen) adsorption behavior on 4:1 PDMS and AG-160-grafted 4:1 PDMS surfaces of Example 2.

[0080] FIG. 28 is an ATR-FTIR spectra of the PDMS samples with PEG grafting reactions carried out in water solution of Example 3.

#### DETAILED DESCRIPTION OF THE INVENTION

##### I. Definitions

[0082] "Polymer" refers to an organic polymer, i.e., a polymer comprising carbon and hydrogen atoms, having a solid bulk polymer matrix. The polymer can also include other atoms such as Si, O, N, P, and S.

[0083] The terms "intrinsic functional group", "intrinsic reactive functional group", "reactive polymerizable functional group", "reactive functional group", and "unreacted functional group" are used interchangeably herein and refer to an unreacted functional group present in the polymer. Such functional groups are present in the monomeric or prepolymer or pre-crosslinked polymer unit(s) of the polymer which react to form a polymer. It should be appreciated that the reactive functional group refers to a functional group that is intrinsically present in the polymer without any additional treatment, e.g., activation, of the polymer. Exemplary reactive functional groups include, but are not limited to, silane, alkene, isocyanate, epoxide, hydroxyl, and the like.

[0084] "Complementary reactive polymerizable functional group" refers to a functional group present in each

polymer component, i.e., monomer or prepolymer or pre-crosslinked polymer, that react with each other to form a polymer.

[0085] "Active functional group" of a surface modifying compound refers to a functional group present in the surface modifying compound which reacts with the reactive functional group of the polymer to form a covalent bond. Exemplary active functional groups include, but are not limited to, hydroxy, alkene, silane, epoxide, isocyanate, and the like.

[0086] "Off ratio polymer" refers to a polymer which is produced from a combination of two or more monomeric or prepolymer or pre-crosslinked polymer units in which at least one monomeric component is present in excess of the other component(s).

[0087] "Property" when referring to the polymer means the polymer's chemical or physical property or both.

[0088] "Biocompatible polymer" refers to a polymer which when exposed to a cell does not significantly change the cell morphology, cell and protein activity, and other cellular functions.

[0089] "Non-specific Binding" (NSB) refers to the binding of ligand to sites other than the receptor. NSB is typically caused by ionic, hydrogen bonding, or other non-covalent interactions. Physical adsorption of proteins on polymer surface is an example of NSB.

[0090] "RTV silicone" refers to room temperature vulcanizing silicone rubber, sometimes referred as poly(dimethylsiloxane), i.e. PDMS.

[0091] "Rotary," when referring to a flow channel, refers to a configuration of the flow channel that allows circulation of a fluid within a confined region or section of the fluid flow channel. Such configuration can be a polygon, such as rectangle, hexagon, octagon, and the like; or, preferably, an ellipse or a circle.

[0092] The terms "microfabricated flow channel," "flow channel," "fluid channel," and "fluid flow channel" are used interchangeably herein and refer to a channel in a microfluidic device in which a fluid, such as gas or, preferably, liquid, can flow through.

[0093] The terms "chromatography column" and "column" are used interchangeably herein and refers to a device or an apparatus which comprises a stationary phase that is capable of separating at least a portion of an analyte in a fluid.

[0094] The term "valve" unless otherwise indicated refers to a configuration in which two channels are separated by an elastomeric segment that can be deflected into or retracted from one of the channels in response to an actuation force applied to the other channel.

##### II. Overview

[0096] Conventional methods of covalently attaching a surface modifying compound to an organic or inorganic substrate typically require activation of the substrate surface. In contrast, the present invention provides a polymer comprising an intrinsic reactive functional group on its surface such that a surface modifying compound can be covalently attached to the polymer surface without the need for a

surface activation step. Thus, one aspect of the present invention provides a surface modified polymer which comprise a bulk polymer matrix comprising a reactive functional group dispersed therein and a polymeric surface (i.e., polymer surface) which is covalently attached to a surface modifying compound.

[0097] The surface modifying compound modifies the physical/chemical characteristics of the polymer surface allowing the surface modified polymer to be used in variety of applications including as chromatography columns, medical devices, chemical assay and/or reaction apparatuses, diagnostic devices, etc. By selecting an appropriate surface modifying compound, the surface modified polymer can be made biocompatible, better solvent resistant, hydrophilic, hydrophobic, wettable, etc. Thus, the surface modified polymers can be used to fabricate a variety of devices, including microfluidic devices such as implantable medical devices, chemical separation devices (e.g., chromatography column), chemical reaction apparatuses and other microfluidic devices.

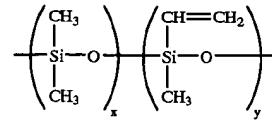
[0098] As stated above, the polymer surface is covalently bonded to the surface modifying compound. This covalent bond is formed by a reaction between a functional group of the surface modifying compound and an intrinsic reactive functional group that is present on the polymer surface. Thus, polymers of the present invention do not require a separate polymer surface activation step, nor the chemical linkers and organic solvents commonly used, thereby significantly reducing the time and cost associated with producing a surface modified polymer. Moreover, since the surface modifying compound is covalently bonded to the polymer surface, problems associated with a non-covalently bound surface modifying compound, such as inability to remain intact and lack of abrasion-resistance during use, are avoided.

[0099] The polymer surface comprises a sufficient amount of the reactive functional group to allow a sufficient amount of the surface modifying compound to be covalently bonded to the polymeric surface. In this manner, a reaction between the reactive functional group of the polymeric surface and a surface modifying compound results in a surface modified polymer which has a substantially different surface physical and/or chemical characteristic than a non-surface modified polymer. Preferably, on the average, polymers that are used to produce surface modified polymers of the present invention comprise at least one reactive functional group per 10,000 monomeric units on the polymer surface. More preferably, polymers of the present invention comprise at least one reactive functional group per 1,000 monomeric units on the polymer surface. And most preferably, polymers of the present invention comprise at least one reactive functional group per 100 monomeric units on the polymer surface. In general, to increase the surface area coverage by a surface modifying compound, macromolecules with the desired functional groups are often used. However, small molecules with the appropriate functional groups can also be used to achieve certain properties such as hydrophilicity.

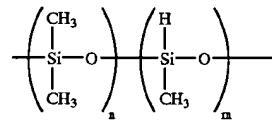
[0100] An illustrative example of a polymer which can be produced to contain a reactive functional group on its bulk polymer matrix and the polymer surface is a silicone polymer, e.g., a polymer produced from GE RTV 615. In particular, GE RTV 615 (i.e., RTV) comprises two compo-

nents: component A which has a vinyl functional group and component B which has a silane functional group. Specifically, components A and B of RTV have the following general structure:

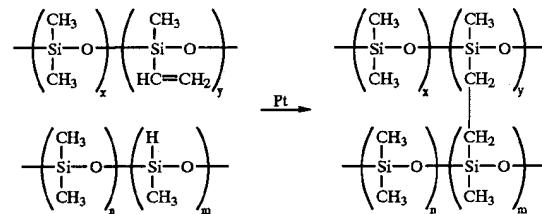
RTV 615A:  
poly(dimethylsiloxane-co-vinylmethylsiloxane)



RTV 615B:  
poly(dimethylsiloxane-co-methylhydrosiloxane)



[0101] A reaction between the vinyl group of RTV 615A and the silane group of RTV 615B catalyzed by a transition metal, e.g., platinum, results in cross-linking reaction to form a polymer according to the following general reaction scheme:



[0102] This reaction (i.e., curing process) is typically a platinum catalyzed hydrosilylation cross-linking reaction in which the vinyl group (C=C) is inserted into the silane group (Si—H).

[0103] For GE RTV 615, 10:1 ratio of component A to component B is typically used or is recommended in the hydrosilylation cross-linking (i.e., curing) reaction. Thus, the resulting polymer has only a minute amount of unreacted silane or vinyl functional groups. As schematically illustrated in FIG. 1A, for GE RTV 615, when the ratio of A:B is >10:1, the resulting silicone polymer comprises an excess unreacted vinyl group, and when the ratio of A:B is <10:1, the resulting silicone polymer comprises an excess unreacted silane group. These functional groups are dispersed within the bulk polymer matrix and are also present on the polymer surface.

[0104] The presence of functional group on the polymer surface allows further reaction with a surface modifying compound directly without the need for a surface activation process. For example, as illustrated in FIG. 1B, reaction between a polymer comprising <10:1 ratio of components A to B with a polyethylene glycol (PEG) results in a covalent bond formation between the silane group and the free hydroxyl group of PEG resulting in a Si—O bond.

[0105] There are a number of other surface modification are possible within the scope of the present invention. Depending on the functional group present on the polymer surface, one can select a surface modifying compound with an appropriate functional group to form a covalent bond. For example, FIG. 1C illustrates two surface modification reactions between a silane functional group of the polymer and hydroxyl or vinyl functional group of a surface modifying compound.

[0106] In addition, FIG. 1C also provides an example of two step process for modifying the silane functional group. In this embodiment, only a small portion of silane group is reacted with a vinyl compound (when  $y > x$ , reaction site amplification is accomplished when the prepolymer is used for grafting). The resulting polymer still comprises unreacted silane group which is reacted with another vinyl group to produce a surface modified polymer comprising two different surface modifying compounds covalently attached to the polymer surface.

#### [0107] Polymers

[0108] Any monomer or a combination of monomers which result in a polymer having unreacted functional groups on the polymer surface can be used in the present invention. Generally, however, three types of polymers are useful in producing surface modified polymer of the present invention: an off-ratio polymers, pre-crosslinked polymers and incompletely crosslinked polymers. Exemplary off-ratio polymers include, but are not limited to, silicone RTV, polyurethane, epoxy, polyurea, unsaturated polyester, etc. Exemplary, pre-crosslinked polymers include, but are not limited to, any vinyl containing elastomer systems, such as, poly(isobutylene isoprene), poly(styrene butadiene), poly(isoprene), poly(butadiene), polychloroprene, vinyl containing rubber gums that can be formulated and milled, etc. The pre-crosslinked rubbers can be surface modified as described herein and vulcanized. Typically, the vulcanization requires a relatively high temperatures; therefore, low temperature reactions, e.g., surface modification, can be carried out prior to the vulcanization reaction. It should be appreciated that the vulcanization reaction reduces or eliminates the reactive functional group within the polymer. Thus, the scope of the present invention includes surface modified polymers in which the reactive functional group within the surface modified polymer is reduced or eliminated by further treating the surface modified polymer, e.g., vulcanizing the surface modified polymer. Exemplary incompletely crosslinked polymers include, but are not limited to, any vinyl containing elastomer systems, such as, poly(isobutylene isoprene), poly(styrene butadiene), poly(isoprene), poly(butadiene), polychloroprene, vinyl containing rubber gums that can be formulated and milled, etc. The crosslinking reaction usually are not complete, therefore, surface modification can be carried utilizing un-reacted functional groups.

[0109] In one embodiment, the polymers of the present invention are prepared by combining at least two different components in which each component includes a complementary reactive functional group. The ratio of each component is selected such that there is an excess of at least one component to provide unreacted function group within the bulk polymer matrix and the polymer surface. Preferably, polymers of the present invention comprise at least one

reactive functional group per 10,000 monomeric units within the bulk polymer matrix. More preferably, polymers of the present invention comprise one reactive functional group per 1,000 monomeric units within the bulk polymer matrix. And most preferably, polymers of the present invention comprise one reactive functional group per 100 monomeric units within the bulk polymer matrix. Preferably, these polymers are produced using an off ratio of each component. Particularly useful off ratio polymers include, but are not limited to:

[0110] silicone polymers which can be produced from monomers comprising a silane and an olefin reactive polymerizable functional groups, e.g., GE's RTV 615, and Dow Coming's Sylgard 184, 182 186;

[0111] polyurethane/polyurea polymers which can be produced from monomers comprising a diisocyanate and an di-alcohol or di-amine reactive polymerizable functional groups, e.g., Synair's 2612020, 2615S 111 and 261S33 or Uniroyal's Vibrathane 504;

[0112] polyisoprene, polybutadiene, polychloroprene which are polymerized from diene monomers, and therefore have one double bond per monomer when polymerized. This double bond on the surface allows the covalent bonding of a surface modifying compound to the polymer. The polymer rubber can then be vulcanized to form a soft elastomer product; and

[0113] styrene butadiene rubber which is produced from an olefin and a diene reactive functional groups of styrene and butadiene, respectively; The double bond presented in the pre-crosslinked and incompletely crosslinked polymer allows the surface of the polymer to be modified.

[0114] Preferable, polymers of the present invention comprise off ratio polymer which comprises complementary reactive groups selected from the group consisting of silane, olefin, hydroxyl, isocyanate, epoxy, and amine. It should be appreciated that the complementary reactive groups are selected such that they are reactive toward each other. For example, if the first monomer component comprises a silane functional group, the second monomer component is selected such that it comprises an olefin, hydroxyl or amine functional group. And if the first monomer component comprises an isocyanante functional group, the second monomer component is selected such that it comprises a hydroxyl or amine functional group, and so forth. In one particular embodiment of the present invention, the polymer is derived from at least two PDMS resins containing silane and olefin functional groups, respectively.

[0115] The amount of each component is selected such that the relative molar ratio of the reactive functional group of one monomeric unit is present in excess of the other(s). In this manner, a significant amount of the reactive functional group of the excess monomer remains unreacted within the bulk polymer matrix and on the polymer surface. Preferably, at least about 1% of the reactive functional group of the excess monomer remains unreacted, more preferably at least about 6%, and most preferably at least about 30%. Alternatively, polymers of the present invention comprise one unreacted reactive functional group per about 10,000 monomeric units, preferably per about 1,000 monomeric units, and more preferably per about 100 monomeric units.

[0116] In one particular embodiment, the polymer is derived from two monomer/prepolymer components. Preferably, the polymer is produced by combining the respective monomer/prepolymer at a relative molar stoichiometric equivalent ratio of from 1:10 to about 1:3, more preferably at a relative molar ratio of from 1:5 to about 1:2, and most preferably at a relative molar ratio of from 1:2 to about 1:1.1.

[0117] Other Suitable Polymer Materials

[0118] Allcock et al., *Contemporary Polymer Chemistry*, 2<sup>nd</sup> Ed. describes elastomers in general as polymers existing at a temperature between their glass transition temperature and liquefaction temperature. Elastomeric materials exhibit elastic properties because the polymer chains readily undergo torsional motion to permit uncoiling of the backbone chains in response to a force, with the backbone chains recoiling to assume the prior shape in the absence of the force. In general, elastomers deform when force is applied, but then return to their original shape when the force is removed. The elasticity exhibited by elastomeric materials can be characterized by a Young's modulus. Elastomeric materials having a Young's modulus of between about 1 Pa to about 1 TPa, more preferably between about 10 Pa to about 100 GPa, more preferably between about 20 Pa to about 1 GPa, more preferably between about 50 Pa to about 10 MPa, and more preferably between about 100 Pa to about 1 MPa are particularly useful in accordance with the present invention. However, elastomeric materials having a Young's modulus outside of these ranges, as well as non-elastomeric materials could also be utilized depending upon the needs of a particular application.

[0119] Surface modified polymers of the present invention can be produced from a wide variety of elastomers, preferably off ratio polymers. In some applications, surface modified polymers of the present invention are produced from an elastomeric polymer such as GE RTV 615, a vinyl-silane crosslinked silicone elastomer. An important requirement for materials which are useful in producing surface modified polymers is the ability to form a polymer with unreacted functional group.

[0120] In one particular embodiment of the present invention, a monolithic polymer is produced from a plurality of layers of elastomers which can be bonded together. In the case of multilayer soft lithography, which is discussed in detail below, layers of elastomer are cured separately and then bonded together. This scheme requires that cured layers possess sufficient reactivity to bond together. The two bonding layers can be of the same type, which are capable of bonding to themselves, or they can be of two different types, which are capable of bonding to each other. Other possibilities include the use an adhesive between layers and the use of thermoset elastomers.

[0121] Given the tremendous diversity of polymer chemistries, precursors, synthetic methods, reaction conditions, and potential additives, there are a huge number of possible polymer, preferably elastomer, systems that could be used to make monolithic elastomeric structures, such as microfluidic devices, discussed below. Variations in the materials used most likely are driven by the need for particular material properties, e.g., stiffness, gas permeability, or temperature stability.

[0122] Common elastomeric polymers include, but are not limited to, polyisoprene, polybutadiene, polychloroprene,

polyisobutylene, poly(styrene-butadiene-styrene), the polyurethanes, and silicones. The following is a non-exclusive list of elastomeric materials which can be utilized in connection with the present invention: polyisoprene, polybutadiene, polychloroprene, polyisobutylene, poly(styrene-butadiene-styrene), the polyurethanes, and silicone polymers; or poly(bis(fluoroalkoxy)phosphazene) (PNF, Eypel-F), poly(carborane-siloxanes) (Dexsil), poly(acrylonitrile-butadiene) (nitrile rubber), poly(1-butene), poly(chlorotrifluoroethylene-vinylidene fluoride) copolymers (Kel-F), poly(ethyl vinyl ether), poly(vinylidene fluoride), poly(vinylidene fluoride-hexafluoropropylene) copolymer (Viton), elastomeric compositions of polyvinylchloride (PVC), polysulfone, polycarbonate, polymethylmethacrylate (PMMA), and polytetrafluoroethylene (Teflon).

[0123] In addition, polymers incorporating materials such as chlorosilanes or methyl-, ethyl-, and phenylsilanes, and polydimethylsiloxane (PDMS) such as Dow Chemical Corp. Sylgard 182, 184 or 186, or aliphatic urethane diacrylates such as, but not limited to, Ebecryl 270 or Irr 245 from UCB Chemical can also be used.

[0124] In some methods, elastomers can also be "doped" with uncrosslinkable polymer chains of the same class. For instance RTV 615 can be diluted with GE SF96-50 Silicone Fluid. This serves to reduce the viscosity of the uncured elastomer and reduces the Young's modulus of the cured elastomer. Essentially, the crosslink-capable polymer chains are spread further apart by the addition of "inert" polymer chains, so this is called "dilution". RTV 615 cures at up to 90% dilution, with a dramatic reduction in Young's modulus.

[0125] Other examples of doping of elastomer material can include the introduction of electrically conducting or magnetic species. Should it be desired, doping with fine particles of material having an index of refraction different than the elastomeric material (i.e. silica, diamond, sapphire) is also contemplated as a system for altering the refractive index of the material. Strongly absorbing or opaque particles can be added to render the elastomer colored or opaque to incident radiation. This can conceivably be beneficial in an optically addressable system.

[0126] While elastomers are preferred materials for fabricating certain surface modified polymer articles of the present invention, e.g., microfluidic devices, non-elastomer based polymer articles are also within the scope of the present invention.

[0127] Surface Modification Compounds

[0128] A wide variety of surface modification compounds can be used to modify the polymer surface depending on a particular characteristic(s) desired. By selecting an appropriate surface modifying compound, the resulting surface modified polymer can be made to be biocompatible, chemically resistance, hydrophilic, hydrophobic, non-sticky, wettable, or combinations thereof. For example, polyethylene glycol (PEG), m-PEG, polyvinyl acetate (PVA), poly(methyl methacrylate) (PMMA), poly(ethylene vinyl acetate), poly(2-hydroxyethyl methacrylate), polyvinylpyrrolidone,  $\text{CH}_2=\text{CH-PEG}$  (i.e., single vinyl terminated PEG), Teflon®, polycarbonate,  $\text{CH}_2=\text{CH-(CH}_2)_n\text{-Teflon}^{\circledR}$  (where  $n = 1$  to 20, i.e., single or double vinyl terminated Teflon®),  $\text{CH}_2=\text{CH-(CH}_2)_n\text{-fluoropolymer}$ ,

and the like can be used to render the polymer biocompatible or solvent resistant. And polymers having non-sticky surfaces can be produced by using a surface modifying compound such as Teflon®, fluoro-containing polymers and copolymers, and the like with vinyl terminal or side groups for chemical solvent resistance and non-sticky surfaces. Moreover, polymers having hydrophilic surfaces can be produced by using a surface modifying compound such as polyvinylpyrrolidone, PVA, PEG, and the like. In addition, polymers having a low surface friction can be produced by using a surface modifying compound such as polyvinylpyrrolidone, PVA, PEG, Teflon®, and the like. Furthermore, polymers useful in chromatography can be produced by using a surface modifying compound which form a stationary phase of a chromatography column. Exemplary surface modifying compounds which can form a stationary phase include, but are not limited to, 1-octadecanol, 1-octadecene, octadecylsilane, octadecyltrichlorosilane, octadecyl isocyanate, trioctadecylsilane, etc. for C<sub>18</sub> grafting (e.g., for stationary phase for C<sub>18</sub> reverse phase chromatography), and corresponding chemicals for C<sub>8</sub>, C<sub>4</sub> or C<sub>2</sub> grafting.

[0129] As stated above, it has been found by the present inventors that the surface of polymers having an intrinsic functional group can be readily modified by covalently attaching a surface modifying compound. Such polymers do not require a separate polymer surface activation step.

[0130] In one particular embodiment of the present invention, the surface modifying compound renders the resulting surface modified polymer biocompatible. Preferably, the biocompatible polymer is derived from covalently attaching a surface modifying compound that is selected from the group consisting of polyethylene glycol, methyl polyethylene glycol, CH<sub>2</sub>=CH-PEG (single vinyl terminated PEG), and CH<sub>2</sub>=CH-(CH<sub>2</sub>)<sub>n</sub>-Teflon (single or double vinyl terminated Teflon).

[0131] In another embodiment, the surface modified polymer is used as a chromatography column by covalently attaching a suitable stationary phase compound to an inner surface of a flow channel, which acts as a chromatography column, that is present in the surface modified polymer. Surface modified polymers comprising a flow channel is discussed in detail below.

[0132] Suitable stationary phase compounds for a particular application are well known to one skilled in the art. For example, useful stationary phase compounds include, but are not limited to, 1-octadecanol, 1-octadecene, octadecylsilane, octadecyltrichlorosilane, octadecyl isocyanate, trioctadecylsilane, etc. for C<sub>18</sub> grafting (e.g., stationary phase for C<sub>18</sub> reverse phase LC), and corresponding compounds for C<sub>8</sub>, C<sub>4</sub> or C<sub>2</sub> stationary phase.

[0133] The stationary phase compounds can be attached to the inner surface of the chromatography column by contacting the stationary phase compound to the inner surface under conditions sufficient to produce a covalent bond. For example, attachment of 1-octadecene to a polymer comprising an intrinsic silane functional group (e.g., Si—H) can be achieved by contacting, e.g., immersing, spraying, or coating, the polymer with 1-octadecene which have a terminal olefin group. The silane group reacts with the olefin group to form an alkyl-silane bond to produce a C-18 stationary phase column. In one particular embodiment of the present invention, the stationary phase compound is useful in form-

ing an open tubular liquid chromatography (i.e., OTLC) column, packed capillary liquid chromatography (i.e., PCLC) column, or mixtures thereof.

[0134] In general, the surface modifying compound can be covalently attached the polymer surface by contacting the surface modifying compound to the polymer surface under conditions sufficient to produce a covalent bond. For example, covalent bond formation between polyethylene glycol and a polymer comprising an intrinsic silane functional group (e.g., Si—H) can be achieved by contacting, e.g., immersing, spraying, or coating, the polymer surface with polyethylene glycol having a terminal hydroxy group. FIG. 2 schematically illustrates three methods for modifying a polymer surface comprising silane functional group. In the first method, silane is reacted with a hydroxyl group to form a silicon-oxygen bond. And the second and third methods of FIG. 2 illustrate formation of a silicon-alkyl bond by reacting silane with an olefin.

[0135] When forming a covalent bond with the intrinsic functional group of the polymer, the surface modifying compound can be in the form of a solution in an inert solvent. Or if the surface modifying compound is a gas, a liquid, or a solid with a melting temperature lower than the reaction temperature (e.g., 200 ° C. or less), it can be used directed without any solvent. When the surface modifying compound is in a solution, the solvent used is relatively inert to the reactive functional group under the reaction conditions. Suitable inert solvents for a particular reactive functional group are well known to one of ordinary skill in the art. For example, suitable inert solvents for a silane reactive functional group include water, methanol, ethanol, isopropanol (IPA), hydrocarbons, ethyl ether, tetrahydrofuran, dimethoxyethane (DME), dimethyl formaldehyde, chloroform, dichloromethane, toluene, xylene, and the like.

[0136] Typically, at least about 1 equiv. of the surface modifying compound is used, preferably at least about 10 equiv., and more preferably at least about 100 equiv. As used herein, the equiv. of the surface modifying compound refers to the equiv. amount of the active functional group of the surface modifying compound relative to the theoretical amount of the intrinsic functional group present on the polymer surface.

[0137] The reaction temperature between the polymer and the surface modifying compound depends on a variety of factors including, the stability of the polymer at an elevated temperature, the concentration and reactivity of the functional groups, the concentration of the catalyst, the stability of the covalent bond that is formed, etc. For example, in reacting PEG to a polymer comprising a silane reactive functional group, the reaction temperature is typically from 20 ° C. to about 200 ° C., preferably from about 50 ° C. to about 120 ° C., and more preferably from 70 ° C. to about 90 ° C.

[0138] The reaction time also depends on a variety of factors such as concentration of the surface modifying compound, the concentration of the catalyst, the reaction temperature, reactivity of the functional groups, and the like. For reacting an allyl-PEG or an olefin, such as 1-octadecene, to a polymer comprising a silane group, the reaction time is typically from about 10 min to about 24 h, preferably from about 20 min to about 4 h, and more preferably from about 30 min to about 2 h.

## [0139] Applications:

[0140] Surface modified polymers of the present invention are useful in a variety of applications, including all conventionally known applications for polymers in general and in particular, in applications where a surface modified polymer is currently used. For example, surface modified polymers of the present invention comprising covalently attached PEG are biocompatible and reduce or prevent protein adsorption and cell attachment. These types of surface modified polymers are particularly useful as biosensors, contact lenses, intraocular lenses, non-fouling food container, heart valves, blood storage containers, blood contacting tubings, cell and protein separation systems, catheters, immunoassays, dialysis membranes, enzyme reactors, solid phase nucleic acid or peptide synthesis, pace maker leads, etc. And chemical resistant surface modified polymers, such as those derived from covalently bonding poly(tetrafluoroethylene) (PTFE or Teflon®), can be used in applications where chemical solvent resistance is desired, such as seals, gaskets, solvent containers, machine parts, tubing, non-sticky surfaces, etc. In addition, surface modified polymers having a low surface friction can be used in a variety of applications where such polymers are desirable, such as in catheter, etc. Moreover, surface modified polymers of the present invention comprising a covalently bound stationary phase compound within the inner surface of the flow channel are useful in chromatography processes and can be used to separate proteins, peptides, and other organic molecules. For example, stationary phase compound bonded surface modified polymers can be used in reverse phase LC, normal phase LC, affinity LC, hydrophobic interaction LC, cation/anion interaction LC, etc. Furthermore, surface modified polymers that are hydrophilic can be used in any application that require a hydrophilic polymer surface. Such hydrophilic surface modified polymers can be produced by covalently bonding polymers comprising an intrinsic functional group with a hydrophilic surface modification compound, such as, poly(2-hydroxyethyl methacrylate), polyvinylpyrrolidone), etc.

## [0141] Microfluidic Devices

[0142] In one particular aspect of the present invention, the surface modified polymers are used in producing microfluidic devices. Use of microfluidic devices has become increasing common recently in fundamental research and biological applications, including, but not limited to, small scale sample preparation, chemical synthesis, drug delivery, biomedical devices, and other applications where a microscale device is desired. While these microfluidic devices have become increasingly popular, one of the limitations for using the microfluidic devices have been the NSB of biomolecules to the polymer surface. Because of the higher surface area to volume ratio in microfluidic environment, the adsorption of reagents in the microfluidic devices onto the surface of the devices result in significant changes in reagent concentration and carryover contamination, etc. Therefore, reducing NSB of the biomolecules onto the microfluidic device surface is of particular importance. Also, many polymers are incompatible in biological systems and many conventional polymers are not chemically resistant to organic solvents, thereby rendering these microfluidic devices unusable in applications involving biological systems and applications involving organic solvents.

[0143] While the surface of conventional polymers can be modified by activating the polymer surface and covalently attaching a surface modifying compound, these processes require a separate surface activation process, which increases the cost and time to fabricate microfluidic devices. In addition, many surface activation processes results in inadequate amount of surface activation, thereby rendering the resulting polymers still incompatible with the environment for which they are intended to be used in.

[0144] Microfluidic devices fabricated from polymers comprising intrinsic functional groups can avoid these shortcomings by producing the polymers such that there is a sufficient amount of intrinsic functional groups for covalently attaching a surface modifying compound. Furthermore, polymers comprising intrinsic functional groups do not require a separate surface activation process, thereby significantly reducing the cost and time for fabrication of microfluidic devices.

[0145] Microfluidic devices can be constructed by any conventional methods known to one skilled in the art, including single and multilayer soft lithography (MLSL) techniques and/or sacrificial-layer encapsulation methods which are disclosed in U.S. patent application Ser. No. 09/605,520, filed Jun. 27, 2000, PCT Publication No. WO 01/01025, and Unger et al. (2000) *Science* 288:113-116, which are incorporated herein by reference in their entirety.

[0146] Microfluidic devices of the present invention comprise a microfabricated flow channel. In addition, microfluidic devices of the present invention can optionally further comprise a variety of plumbing components (e.g., pumps, valves, and connecting channels) for flowing fluids (i.e., gas or, preferably, liquid) such as reagents, solvents, and samples. The microfluidic devices can also comprise an array of reservoirs for storing reaction reagents (e.g., solvents, samples, eluents, and other reagents can each be stored in a different reservoir). The flow channel can be located within the bulk polymer matrix or it can be at least partially enclosed by a combination of a solid support and the bulk polymer matrix. Thus, the flow channel comprises an inner surface, at least a portion of which is defined by the bulk polymer matrix.

[0147] Preferably, the surface modifying compound is covalently bonded to any surface in which a surface modification is desired, including the exterior surface of the bulk polymer matrix and/or the inner surface of the flow channel. The microfluidic devices can comprise a plurality of microfabricated flow channels that are interconnected to suit a variety of applications. The flow channel can also comprise a window to allow optical interrogation.

[0148] Microfluidic devices require a smaller sample size and the amount of reagent(s). It also increases reagent exchange rate and the speed of sample analysis or preparation. In addition, the microfluidic devices provide parallelization: many flow channels can be built on the same substrate, e.g., polymer. This allows analysis, preparation, and/or synthesis of a plurality of diverse samples simultaneously. All these advantages result in high speed and high throughput sample preparation, analysis, and/or synthesis regimes.

[0149] Basic features of the microfluidic devices

[0150] The microfluidic devices of the present invention comprise a basic "flow channel" structure. The term "flow

channel", "fluid channel", or "microfabricated flow channel" refers to recess in a structure which can contain a flow of fluid such as gas or, preferably, liquid. Preferably, the surface modifying compound is covalently attached to the inner surface of the flow channel(s).

[0151] The microfluidic devices can also comprise a control channel. The control channel functions as the plumbing components of the microfluidic devices, e.g., valves and pumps, which is comprised of one or more valves. A "valve" refers to a segment of bulk polymer matrix, preferably an elastomeric segment, that separates the flow channel and the control channel. The valve can be deflected into or retracted from the flow channel in response to an actuation force applied to the control channel.

[0152] The inner surface of control channels can also be covalently bonded to the surface modifying compound. However, because the control channels typically do not come in contact with incompatible environmental conditions, it is not necessary to attach a surface modifying compound to inner surfaces of the control channels.

[0153] In some applications, microfabricated flow channels are cast on a chip (e.g., a elastomeric chip). Fluid channels are formed by bonding the chip to a flat substrate (e.g., a glass cover slip or another polymer) which seals the channel. Thus, one side of the fluid channel is provided by the flat substrate. Typically, the surface modifying compound is attached to the inner surface of the fluid channel. However, it should be appreciated that any microfluidic device surface which may come in contact with a reagent or a sample fluid is covalently bonded to a surface modifying compound. For example, for an implantable microfluidic device, preferably the entire polymer surface, including the inner fluid flow surface and exterior surface of the bulk polymer matrix, is covalently attached to a biocompatible surface modifying compound, thereby reducing or eliminating any potential interaction between the host (e.g., patient) and the device.

[0154] As stated above, the plumbing components can also be microfabricated within the microfluidic device. Thus, microfluidic devices can include an integrated flow cell in which a plurality of fluid channels are present. In addition, microfluidic devices can also include fluidic components, such as micro-pumps, micro-valves, and connecting channels, for controlling the flow of fluids into and out of the flow cell. Alternatively, microfluidic devices of the present invention can utilize other plumbing devices. See, for example, Zdeblick et al., *A Microminiature Electric-to-Fluidic Valve*, Proceedings of the 4th International Conference on Solid State Transducers and Actuators, 1987; Shoji et al., *Smallest Dead Volume Microvalves for Integrated Chemical Analyzing Systems*, Proceedings of Transducers '91, San Francisco, 1991; and Vieider et al., *A Pneumatically Actuated Micro Valve with a Silicon Rubber Membrane for Integration with Fluid Handling Systems*, Proceedings of Transducers '95, Stockholm, 1995, all of which are incorporated herein by reference in their entirety.

[0155] At least some of the components of the microfluidic devices of the present invention are microfabricated. Employment of microfabricated fluid channels and/or microfabricated plumbing components significantly reduce the dead volume and decrease the amount of time needed to exchange reagents, which in turn increase the throughput.

Microfabrication refers to feature dimensions on the micron level, with at least one dimension of the microfabricated structure being less than 1000  $\mu\text{m}$ . In some microfluidic devices, only the fluid channels are microfabricated. In some microfluidic devices, in addition to the fluid channels, the valves, pumps, and connecting channels are also microfabricated. Unless otherwise specified, the discussion below of microfabrication is applicable to production of all microfabricated components of the microfluidic devices (e.g., the fluid channels, valves, pumps, and connecting channels).

[0156] As stated above, various materials can be used to produce the microfluidic devices. Preferably, microfluidic devices of the present invention comprise an elastomeric polymer. In some embodiments, microfluidic devices are integrated (i.e., monolithic) microstructures that are made out of various layers of elastomer bonded together. By bonding these various elastomeric layers together, the recesses extending along the various elastomeric layers form fluid channels and/or control channels through the resulting monolithic, integral elastomeric structure.

[0157] In general, the microfabricated structures (e.g., fluid channels, pumps, valves, and connecting channels) have widths of about 0.01 to 1000 microns, and a width-to-depth ratios of between 0.1:1 to 100:1. Preferably, the width is in the range of 10 to 200 microns, a width-to-depth ratio of 3:1 to 15:1.

#### [0158] Basic Methods of Microfabrication

[0159] Various methods can be used to produce the microfabricated components of the microfluidic devices of the present invention. Fabrication of the microchannels, such as fluid flow channels, valves, and pumps, can be performed as described in the above incorporated U.S. patent application Ser. No. 09/605,520, filed Jun. 27, 2000, PCT Publication No. WO 01/01025, and Unger et al. (2000) *Science* 288:113-116.

[0160] In some methods, as illustrated in FIGS. 3-9B, pre-cured elastomer layers are assembled and bonded to produce a flow channel. As illustrated in FIG. 3, a first micro-machined mold 10 is provided. Micro-machined mold 10 can be fabricated by a number of conventional silicon processing methods including, but not limited to, photolithography, ion-milling, and electron beam lithography. The micro-machined mold 10 has a raised line or protrusion 11 extending therealong. A first elastomeric layer 20 is cast on top of mold 10 such that a first recess 21 can be formed in the bottom surface of elastomeric layer 20, (recess 21 corresponding in dimension to protrusion 11), as shown.

[0161] As can be seen in FIG. 4, a second micro-machined mold 12 having a raised protrusion 13 extending therealong is also provided. A second elastomeric layer 22 is cast on top of mold 12, as shown, such that a recess 23 can be formed in its bottom surface corresponding to the dimensions of protrusion 13.

[0162] As can be seen in the sequential steps illustrated in FIGS. 5 and 6, second elastomeric layer 22 is then removed from mold 12 and placed on top of first elastomeric layer 20. As can be seen, recess 23 extending along the bottom surface of second elastomeric layer 22 forms a flow channel 32.

[0163] Referring to FIG. 7, the separate first and second elastomeric layers 20 and 22 (FIG. 6) are then bonded together to form an integrated (i.e., monolithic) elastomeric structure 24.

[0164] As can be seen in the sequential step of FIGS. 8 and 9A, elastomeric structure 24 is then removed from mold 10 and positioned on top of a planar substrate 14. As can be seen in FIG. 9A and 9B, when elastomeric structure 24 has been sealed at its bottom surface to planar substrate 14, recess 21 forms a flow channel 30.

[0165] The present elastomeric structures can form a reversible hermetic seal with nearly any smooth planar substrate. An advantage to forming a seal this way is that the elastomeric structures can be peeled up, washed, and re-used. In some microfluidic devices, planar substrate 14 is glass. A further advantage of using glass is that glass is transparent, allowing optical interrogation of elastomer channels and reservoirs. Alternatively, the elastomeric structure can be bonded onto a flat elastomer layer by the same method as described above, forming a permanent and high-strength bond. This can prove advantageous when higher back pressure is used.

[0166] In some methods, microfabrication involves curing each layer of elastomer "in place" (FIGS. 10 to 20). In these methods, fluid flow and control channels are defined by first patterning sacrificial layer on the surface of an elastomeric layer (or other substrate, which can include glass) leaving a raised line of sacrificial layer where a channel is desired. Next, a second layer of elastomer is added thereover and a second sacrificial layer is patterned on the second layer of elastomer leaving a raised line of sacrificial layer where a channel is desired. A third layer of elastomer is deposited thereover. Finally, the sacrificial layer is removed by dissolving it out of the elastomer with an appropriate solvent, with the voids formed by removal of the sacrificial layer becoming the flow channels passing through the microfluidic device.

[0167] Referring first to FIG. 10, a planar substrate 40 is provided. A first elastomeric layer 42 is then deposited and cured on top of planar substrate 40. Referring to FIG. 11, a first sacrificial layer 44A is then deposited over the top of elastomeric layer 42. Referring to FIG. 12, a portion of sacrificial layer 44A is removed such that only a first line of sacrificial layer 44B remains as shown. Referring to FIG. 13, a second elastomeric layer 46 is then deposited over the top of first elastomeric layer 42 and over the first line of sacrificial layer 44B as shown, thereby encasing first line of sacrificial layer 44B between first elastomeric layer 42 and second elastomeric layer 46. Referring to FIG. 14, elastomeric layers 46 is then cured on layer 42 to bond the layers together to form a monolithic elastomeric substrate 45.

[0168] Referring to FIG. 15, a second sacrificial layer 48A is then deposited over elastomeric structure 45. Referring to FIG. 16, a portion of second sacrificial layer 48A is removed, leaving only a second sacrificial layer 48B on top of elastomeric structure 45 as shown. Referring to FIG. 17, a third elastomeric layer 50 is then deposited over the top of elastomeric structure 45 (comprised of second elastomeric layer 42 and first line of sacrificial layer 44B) and second sacrificial layer 48B as shown, thereby encasing the second line of sacrificial layer 48B between elastomeric structure 45 and third elastomeric layer 50.

[0169] Referring to FIG. 18, third elastomeric layer 50 and elastomeric structure 45 (comprising first elastomeric layer 42 and second elastomeric layer 46 bonded together) is then bonded together forming a monolithic elastomeric structure 47 having sacrificial layers 44B and 48B passing therethrough as shown. Referring to FIG. 19, sacrificial layers 44B and 48B are then removed (for example, by dissolving in a solvent) such that a first flow channel 60 and a second flow channel 62 are provided in their place, passing through elastomeric structure 47 as shown. Lastly, referring to FIG. 20, planar substrate 40 can be removed from the bottom of the integrated monolithic structure.

#### [0170] Multilayer Construction

[0171] Soft lithographic bonding can be used to construct an integrated system which contains multiple fluid flow channels. A heterogenous bonding can be used in which different layers are of different chemistries. For example, the bonding process used to bind respective elastomeric layers together can comprise bonding together two layers of RTV 615 silicone. RTV 615 silicone is a two-part addition-cure silicone rubber. Part A contains vinyl groups and catalyst; part B contains silane (Si—H) groups. The conventional ratio for RTV 615 is 10A:1B. For bonding, one layer can be made with 30A:1B (i.e. excess vinyl groups) and the other with 3A:1B (i.e. excess silane groups). Each layer is cured separately. When the two layers are brought into contact and heated at elevated temperature, they bond irreversibly forming a monolithic elastomeric substrate.

[0172] A homogenous bonding can also be used in which all layers are of the same chemistry. For example, elastomeric structures are formed utilizing Sylgard 182, 184 or 186, or aliphatic urethane diacrylates such as (but not limited to) Ebecryl 270 or Irr 245 from UCB Chemical. For example, two-layer elastomeric structures were fabricated from pure acrylated Urethane Ebe 270. A thin bottom layer was spin coated at 8000 rpm for 15 seconds at 170 °C. The top and bottom layers were initially cured under ultraviolet light for 10 minutes under nitrogen utilizing a Model ELC 500 device manufactured by Electrolite corporation. The assembled layers were then cured for an additional 30 minutes. Reaction was catalyzed by a 0.5% vol/vol mixture of Irgacure 500 manufactured by Ciba-Geigy Chemicals. The resulting elastomeric material exhibited moderate elasticity and adhesion to glass.

[0173] In some applications, two-layer elastomeric structures were fabricated from a combination of 25% Ebe 270/50% Irr245/25% isopropyl alcohol for a thin bottom layer, and pure acrylated Urethane Ebe 270 as a top layer. The thin bottom layer was initially cured for 5 min, and the top layer initially cured for 10 minutes, under ultraviolet light under nitrogen utilizing a Model ELC 500 device manufactured by Electrolite corporation. The assembled layers were then cured for an additional 30 minutes. Reaction was catalyzed by a 0.5% vol/vol mixture of Irgacure 500 manufactured by Ciba-Geigy Chemicals. The resulting elastomeric material exhibited moderate elasticity and adhered to glass.

[0174] Where encapsulation of sacrificial layers is employed to fabricate the elastomer structure as described above in FIGS. 10-20, bonding of successive elastomeric layers can be accomplished by pouring uncured elastomer over a previously cured elastomeric layer and any sacrificial

material patterned thereupon. Bonding between elastomer layers occurs due to interpenetration and reaction of the polymer chains of an uncured elastomer layer with the polymer chains of a cured elastomer layer. Subsequent curing of the elastomeric layer creates a monolithic elastomeric structure in which a bond is formed between the elastomeric layers.

[0175] Referring to the first method of FIGS. 3 to 9B, first elastomeric layer 20 can be created by spin-coating an RTV mixture on microfabricated mold 12 at 2000 rpm for 30 seconds yielding a thickness of approximately 40 microns. Second elastomeric layer 22 can be created by spin-coating an RTV mixture on microfabricated mold 11. Both layers 20 and 22 can be separately baked or cured at about 80° C. for 1.5 hours. The second elastomeric layer 22 can be bonded onto first elastomeric layer 20 at about 80° C. for about 1.5 hours.

[0176] Micromachined molds 10 and 12 can be patterned sacrificial layer on silicon wafers. In an exemplary aspect, a Shipley SJR 5740 sacrificial layer was spun at 2000 rpm patterned with a high resolution transparency film as a mask and then developed yielding an inverse channel of approximately 10 microns in height. When baked at approximately 200 ° C. for about 30 minutes, the sacrificial layer reflows and the inverse channels become rounded. Optionally, the molds can be treated with trimethylchlorosilane (TMCS) vapor for about a minute before each use in order to prevent adhesion of silicone rubber.

#### [0177] Dimensions of the Microfabricated Structures

[0178] Some channels (30, 32, 60 and 62) preferably have width-to-depth ratios of about 10:1. A non-exclusive list of other ranges of width-to-depth ratios in accordance with the present invention is 0.1:1 to 100:1, more preferably 1:1 to 50:1, more preferably 2:1 to 20:1, and most preferably 3:1 to 15:1. In an exemplary aspect, channels 30, 32, 60 and 62 have widths of about 1 to about 1000 microns. A non-exclusive list of other ranges of widths of channels in accordance with the present invention is about 0.01 to about 1000 microns, more preferably about 0.05 to about 1000 microns, more preferably about 0.2 to about 500 microns, more preferably about 1 to about 250 microns, and most preferably about 10 to about 200 microns. Exemplary channel widths include 0.1  $\mu$ m, 1  $\mu$ m, 2  $\mu$ m, 5  $\mu$ m, 10  $\mu$ m, 20  $\mu$ m, 30  $\mu$ m, 40  $\mu$ m, 50  $\mu$ m, 60  $\mu$ m, 70  $\mu$ m, 80  $\mu$ m, 90  $\mu$ m, 100  $\mu$ m, 110  $\mu$ m, 120  $\mu$ m, 130  $\mu$ m, 140  $\mu$ m, 150  $\mu$ m, 160  $\mu$ m, 170  $\mu$ m, 180  $\mu$ m, 190  $\mu$ m, 200  $\mu$ m, 210  $\mu$ m, 220  $\mu$ m, 230  $\mu$ m, 240  $\mu$ m, and 250  $\mu$ m.

[0179] Channels 30, 32, 60, and 62 have depths of about 1 to about 100 microns. A non-exclusive list of other ranges of depths of channels in accordance with the present invention is about 0.01 to about 1000 microns, more preferably about 0.05 to about 500 microns, more preferably about 0.2 to about 250 microns, and more preferably about 1 to about 100 microns, more preferably 2 to 20 microns, and most preferably 5 to 10 microns. Exemplary depths include including 0.01  $\mu$ m, 0.02  $\mu$ m, 0.05  $\mu$ m, 0.1  $\mu$ m, 0.2  $\mu$ m, 0.5  $\mu$ m, 1  $\mu$ m, 2  $\mu$ m, 3  $\mu$ m, 4  $\mu$ m, 5  $\mu$ m, 7.5  $\mu$ m, 10  $\mu$ m, 12.5  $\mu$ m, 15  $\mu$ m, 17.5  $\mu$ m, 20  $\mu$ m, 30  $\mu$ m, 40  $\mu$ m, 50  $\mu$ m, 75  $\mu$ m, 100  $\mu$ m, 150  $\mu$ m, 200  $\mu$ m, and 250  $\mu$ m.

[0180] The channels are not limited to these specific dimension ranges and examples given above, and can vary

in width in order to affect the magnitude of force required to deflect the elastomeric segment. For example, extremely narrow channels having a width on the order of 0.01  $\mu$ m can be useful in a variety of applications. Elastomeric structures which include portions having channels of even greater width than described above are also contemplated by the present invention, and examples of applications of utilizing such wider channels include fluid reservoir and mixing channel structures.

[0181] Elastomeric layer 22 can be cast thick for mechanical stability. In an exemplary embodiment, layer 22 is about 50 microns to several centimeters thick, and more preferably approximately 4 mm thick. A non-exclusive list of ranges of thickness of the elastomer layer in accordance with other embodiments of the present invention is between about 0.1 micron to about 10 cm, 1 micron to 5 cm, 10 microns to 2 cm, and 100 microns to 10 mm.

[0182] Accordingly, elastomeric segment 25 of FIG. 9B separating channels 30 and 32 has a typical thickness of between about 0.01 and about 1000 microns, more preferably about 0.05 to about 500 microns, still more preferably about 0.2 to about 250, yet more preferably about 1 to about 100 microns, still yet more preferably about 2 to about 50 microns, and most preferably about 5 to about 40 microns. As such, the thickness of elastomeric layer 22 is about 100 times the thickness of elastomeric layer 20. Exemplary elastomeric segment thicknesses include 0.01  $\mu$ m, 0.02  $\mu$ m, 0.03  $\mu$ m, 0.05  $\mu$ m, 0.1  $\mu$ m, 0.2  $\mu$ m, 0.3  $\mu$ m, 0.5  $\mu$ m, 1  $\mu$ m, 2  $\mu$ m, 3  $\mu$ m, 5  $\mu$ m, 7.5  $\mu$ m, 10  $\mu$ m, 12.5  $\mu$ m, 15  $\mu$ m, 17.5  $\mu$ m, 20  $\mu$ m, 22.5  $\mu$ m, 25  $\mu$ m, 30  $\mu$ m, 40  $\mu$ m, 50  $\mu$ m, 75  $\mu$ m, 100  $\mu$ m, 150  $\mu$ m, 200  $\mu$ m, 250  $\mu$ m, 300  $\mu$ m, 400  $\mu$ m, 500  $\mu$ m, 750  $\mu$ m, and 1000  $\mu$ m.

[0183] Similarly, first elastomeric layer 42 can have a preferred thickness about equal to that of elastomeric layer 20 or 22; second elastomeric layer 46 can have a preferred thickness about equal to that of elastomeric layer 20; and third elastomeric layer 50 can have a preferred thickness about equal to that of elastomeric layer 22.

#### [0184] Operation of the microfabricated components

[0185] FIGS. 9B and 9C together show the closing of a flow channel by pressurizing a control channel. FIG. 9B (a front sectional view cutting through flow channel 32 in corresponding FIG. 9A) shows an open flow channel 30. And FIG. 9C shows flow channel 30 closed by pressurization of the control channel 32.

[0186] Referring to FIG. 9B, the flow channel 30 and the control channel 32 are shown. Elastomeric segment 25 separates the channels, forming the top of the flow channel 30 and the bottom of the control channel 32. As can be seen, flow channel 30 is "open".

[0187] As can be seen in FIG. 9C, pressurization of control channel 32 (either by gas or liquid introduced therein) causes elastomeric segment 25 to deflect downward, thereby pinching off flow F passing through flow channel 30. Accordingly, by varying the pressure in control channel 32, a linearly actuatable valve system is provided such that flow channel 30 can be opened or closed by moving elastomeric segment 25 as desired. (For illustration purposes only, flow channel 30 in FIG. 9C is shown in a "mostly closed" position, rather than a "fully closed" position).

[0188] It is to be understood that exactly the same valve opening and closing methods can be achieved with channels 60 and 62. Since such valves are actuated by moving the roof of the channels themselves (i.e., moving elastomeric segment 25), valves and pumps produced by this technique have a truly zero dead volume, and switching valves made by this technique have a dead volume approximately equal to the active volume of the valve, for example, about  $100 \times 100 \times 10 \mu\text{m} = 100 \text{ pL}$ . Such dead volumes and areas consumed by the moving elastomeric segment are approximately two orders of magnitude smaller than known conventional microvalves. Smaller and larger valves and switching valves are contemplated in the present invention, and a non-exclusive list of ranges of dead volume includes 1 aL to 1  $\mu\text{L}$ , 100 aL to 100 nL, 1 fL, to 10 nL, 100 fL to 1 nL, and 1 pL to 100 pL.

[0189] The extremely small volumes capable of being delivered by pumps and valves in accordance with the present invention represent a substantial advantage. Specifically, the smallest known volumes of fluid capable of being manually metered is around 0.1  $\mu\text{L}$ . The smallest known volumes capable of being metered by automated systems is about ten-times larger (1  $\mu\text{L}$ ). Utilizing pumps and valves of the present invention, volumes of liquid of 10 nL or smaller can routinely be metered and dispensed. The accurate metering of extremely small volumes of fluid enabled by the present invention would be extremely valuable in a large number of biological applications, including medical devices, diagnostic tests, sample preparations, sample separations, and sample analysis (i.e., assays).

[0190] FIGS. 21a and 21b illustrate valve opening vs. applied pressure for a 100  $\mu\text{m}$  wide flow channel 30 and a 50  $\mu\text{m}$  wide control channel 32. The elastomeric segment of this device was formed by a layer of General Electric Silicones RTV 615 having a thickness of approximately 30  $\mu\text{m}$  and a Young's modulus of approximately 750 kPa. FIGS. 21a and 21b show the extent of opening of the valve to be substantially linear over most of the range of applied pressures.

[0191] Air pressure was applied to actuate the elastomeric segment of the device through a 10 cm long piece of plastic tubing having an outer diameter of 0.025" connected to a 25 mm piece of stainless steel hypodermic tubing with an outer diameter of 0.025" and an inner diameter of 0.013". This tubing was placed into contact with the control channel by insertion into the elastomeric block in a direction normal to the control channel. Air pressure was applied to the hypodermic tubing from an external LHDA miniature solenoid valve manufactured by Lee Co.

[0192] The response of valves of the present invention is substantially linear over a large portion of its range of travel, with minimal hysteresis. While valves and pumps do not require linear actuation to open and close, linear response does allow valves to more easily be used as metering devices. In some applications, the opening of the valve is used to control flow rate by being partially actuated to a known degree of closure. Linear valve actuation makes it easier to determine the amount of actuation force required to close the valve to a desired degree of closure. Another benefit of linear actuation is that the force required for valve actuation can be easily determined from the pressure in the flow channel. If actuation is linear, increased pressure in the flow channel can be countered by adding the same pressure (force per unit area) to the actuated portion of the valve.

[0193] Control and Pump Systems

[0194] FIGS. 22A and 22B show views of a single on/off valve (e.g., flow control system), identical to the systems set forth above, (for example in FIG. 9A). FIGS. 23A and 23B show a peristaltic pumping system (e.g., a material delivery system) comprised of a plurality of the single addressable on/off valves as seen in FIGS. 22A and 22B, but networked together. FIG. 24 is a graph showing experimentally achieved pumping rates vs. frequency for the peristaltic pumping system of FIGS. 23A and 23B.

[0195] Referring first to FIGS. 22A and 22B, a schematic of channels 30 and 32 is shown. Flow channel 30 preferably has a fluid (or gas) flow F passing therethrough. Control channel 32, which crosses over flow channel 30, is pressurized such that elastomeric segment 25 separating the channels is depressed into the path of flow channel 30, shutting off the passage of flow F therethrough, as described above.

[0196] Referring to FIGS. 23A and 23B, a system for peristaltic pumping is provided, as follows. A flow channel 30 has a plurality of generally parallel control channels 32A, 32B and 32C passing thereover. By pressurizing control line 32A, flow F through flow channel 30 is shut off under elastomeric segment 25A at the intersection of control line 32A 30 and flow channel 30. Similarly, (but not shown), by pressurizing control line 32B, flow F through flow channel 30 is shut off under elastomeric segment 25B at the intersection of control line 32B and flow channel 30, etc. Each of control lines 32A, 32B, and 32C is separately addressable. Therefore, peristalsis can be actuated by the pattern of actuating 32A and 32C together, followed by 32A, followed by 32A and 32B together, followed by 32B, followed by 32B and C together, etc. This corresponds to a successive "101, 100, 110, 010, 011, 001" pattern, where "0" indicates "valve open" and "1" indicates "valve closed." This peristaltic pattern is also known as a 120° pattern (referring to the phase angle of actuation between three valves). Other peristaltic patterns are equally possible, including 60° and 90° patterns.

[0197] Using this process, a pumping rate of 2.35 nL/s was measured by measuring the distance traveled by a column of water in thin (0.5 mm i.d.) tubing; with  $100 \times 100 \times 10 \mu\text{m}$  valves under an actuation pressure of 40 kPa. As shown in FIG. 24, the pumping rate increased with actuation frequency until approximately at about 75 Hz, and from about 75 Hz to above 200 Hz the pumping rate was nearly constant. The valves and pumps are also quite durable and the elastomeric segment, control channels, or both have not been observed to fail. Moreover, none of the valves in the peristaltic pump described herein shows any sign of wear or fatigue after more than 4 million actuations.

[0198] Variations

[0199] In some applications, microfluidic devices produced from the surface modified polymer, such as microfluidic chromatography apparatuses, utilize microfluidics based on conventional micro-electro-mechanical system (MEMS) technology. Methods of producing conventional MEMS microfluidic devices, e.g., by bulk micro-machining and surface micro-machining, have been described. See, for example, Terry et al., *A Gas Chromatographic Air Analyzer Fabricated on a Silicon Wafer*, IEEE Trans. on Electron Devices, v. ED-26, pp. 1880-1886, 1979; and Berg et al.,

*Micro Total Analysis Systems*, New York, Kluwer, 1994, all of which are incorporated herein by reference in their entirety.

[0200] Bulk micro-machining is a subtractive fabrication method whereby single crystal silicon is lithographically patterned and then etched to form three-dimensional structures. For example, bulk micromachining technology, which includes the use of glass wafer processing, silicon-to-glass wafer bonding, has been commonly used to fabricate individual microfluidic components. This glass-bonding technology has also been used to fabricate microfluidic devices.

[0201] Surface micro-machining is an additive method where layers of semiconductor-type materials such as polysilicon, silicon nitride, silicon dioxide, and various metals are sequentially added and patterned to make three-dimensional structures. Surface micromachining technology can be used to fabricate individual fluidic components as well as microfluidic systems with on-chip electronics. In addition, unlike bonded-type devices, hermetic channels can be built in a relatively simple manner using channel walls made of polysilicon (see, e.g., Webster et al., *Monolithic Capillary Gel Electrophoresis Stage with On-Chip Detector*, in International Conference on Micro Electromechanical Systems, MEMS '96, pp. 491-496, 1996), silicon nitride (see, e.g., Mastrangelo et al., *Vacuum-Sealed Silicon Micromachined Incandescent Light Source*, in Intl. Electron Devices Meeting, IDEM '89, pp. 503-506, 1989), and silicon dioxide.

[0202] In some applications, electrokinetic flow based microfluidics can be employed. Briefly, these systems direct fluid flow within an interconnected channel and/or chamber containing structure through the application of electrical fields to the fluid. The electrokinetic systems concomitantly regulate voltage gradients applied across at least two intersecting channels. Such systems are described, e.g., in WO 96/04547 and U.S. Pat. No. 6,107,044.

[0203] An exemplary electrokinetic flow based microfluidic device can have a body structure which includes at least two intersecting channels or fluid conduits, e.g., interconnected, enclosed chambers, which channels include at least three unintersected termini. The intersection of two channels refers to a point at which two or more channels are in fluid communication with each other, and encompasses "T" intersections, cross intersections, "wagon wheel" intersections of multiple channels, or any other channel geometry where two or more channels are in such fluid communication. An unintersected terminus of a channel is a point at which a channel terminates not as a result of that channel's intersection with another channel, e.g., "T" intersection.

[0204] In some electrokinetic flow based microfluidic devices, at least three intersecting channels having at least four unintersected termini are present. In a basic cross channel structure, where a single horizontal channel is intersected and crossed by a single vertical channel, controlled electrokinetic transport operates to direct reagent flow through the intersection, by providing constraining flows from the other channels at the intersection.

[0205] Simple electrokinetic flow of this reagent across the intersection could be accomplished by applying a voltage gradient across the length of the horizontal channel, i.e., applying a first voltage to the left terminus of this channel, and a second, lower voltage to the right terminus of this channel, or by allowing the right terminus to float (applying no voltage).

## EXAMPLES

[0206] Additional objects, advantages, and novel features of this invention will become apparent to those skilled in the art upon examination of the following examples thereof, which are not intended to be limiting.

### Example 1

[0207] A 3:1 (A:B) GE RTV 615 silicone mixture was cured at 80° C. in a convection oven for 3 hrs. The silicone sample was then immersed in a molten PEG sample (molecular weight 1,000, Polysciences, Inc.) at 80° C. for 24 hrs. The surface treated samples were then repeatedly washed with high purity deionized (i.e., DI) water for 2 hrs. The resulting samples were incubated with conjugated protein solutions for 2 hrs at room temperature. The protein solutions used were: insulin-FITC (1 mg/mL), BSA-FITC (1 mg/mL), IgG-FITC (1 mg/mL) and Streptavidin-Cy3 (0.2 mg/mL). The PEG grafted silicone samples were then washed with DI water gently three times. Fluorescence microscope was used to record the fluorescence intensity of the samples. The settings of the microscope were at such that the intensity is linearly proportional to the amount of the residue protein on the surface. As shown in FIG. 25, results indicate that the PEG treated surface reduces the adsorption of proteins from 95% (insulin-FITC) to about 75% (BSA-FITC).

### Example 2

[0208] A 4:1 GE RTV silicone mixture was cured at 80° C. in a convection oven for 3 hrs. The excess hydrosilane groups from RTV 615B on the surface were utilized for polyethylene glycol grafting. An allyl-derived PEG, polyoxyethylene mono-allylether  $\text{CH}_2=\text{CHCH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_{16}-\text{H}$  (AG-160) from Kowa was used for surface grafting. Platinum chloride catalyst,  $\text{H}_2\text{PtCl}_6$  (Sigma-Aldrich), was used to catalyze the hydrosilylation reaction. The catalyst concentration used was in the range of 100-1000 ppm (weight %) and the reaction time at 80° C. ranged from several minutes to hours, depending on the catalyst concentration. As expected, the higher the catalyst concentration, the shorter the reaction time was needed to achieve a desired degree of reaction. The molten AG-160 was used to carry out the reactions. The samples were then washed thoroughly with methanol and DI water in ultrasonic bath. The samples were then dried at 80° C. for 2 hrs. After the cleaning and drying, the top  $\sim 1 \mu\text{m}$  of the PDMS samples were investigated by attenuated total reflection Fourier transfer infrared spectroscopy (ATR-FTIR). FIG. 26 is an ATR-FTIR spectra of the surfaces of the 4:1 treated PDMS samples as a function of reaction time. The IR absorption peak at  $2160 \text{ cm}^{-1}$  is characteristic of the Si-H functional group and the intensity of this peak is linearly proportional to the concentration of the Si-H group on the top  $\sim 1 \mu\text{m}$  surface of the samples. The hydrosilylation reaction between Si-H and  $\text{CH}_2=\text{CH}$ -groups results in a decrease in the absorption intensity of the Si-H peak. As illustrated in FIG. 26, the Si-H peak intensity decreases and disappears with increasing reaction time. This decrease in the Si-H peak shows the conversion of the Si-H functional groups to Si-C bonds that are due to the grafting of AG-160. Additional evidences for the PEG grafting is the peaks at  $1330$  and  $2880 \text{ cm}^{-1}$  which is due to the  $-\text{O}-\text{C}-\text{H}$  absorption band.

[0209] ATR-FTIR was also used to study protein absorption behavior of PEG grafted PDMS surfaces. The PDMS samples were incubated with 0.15 mg/mL fibrinogen in PBS (1x) solution for 24 h. The samples were then washed with DI water and then dried. FIG. 27 shows an ATR-FTIR spectra of protein (fibrinogen) adsorption behavior on 4:1 PDMS and AG-160-grafted 4:1 PDMS surfaces. The most characteristic IR peaks for proteins are amide I ( $\sim 1645\text{ cm}^{-1}$ ) and amide II ( $\sim 1550\text{ cm}^{-1}$ ). Since either PDMS or PEG has absorption peaks in these regions, they can be used for quantitative estimation of proteins adsorbed on the surface. It is clear from the spectra that there is less protein adsorbed on the PEG (AG-160) grafted surface.

### Example 3

[0210] A 4:1 GE RTV 615 silicone mixture was cured at 80° C. in a convection oven for 3 hrs. The excess hydroxyl groups from RTV 615B on the surface were utilized for polyethylene glycol grafting. Allyl-derived PEG, polyoxyethylene CH<sub>2</sub>=CHCH<sub>2</sub>O(CH<sub>2</sub>CH<sub>2</sub>O)<sub>16</sub>-H (AG-160) from Kowa was used for surface grafting. The catalyst concentration is in the range of 100-1000 ppm (weight %) and the reaction time at 80° C. was from several minutes to several hours. Aqueous AG-160 solution (10 wt % of AG-160) was used to carry out the grafting reactions. FIG. 28 is an ATR-FTIR spectra of the PDMS samples with PEG grafting reactions carried out in water solution. The reaction trend is very similar to that of Example 1, but higher intensity of hydroxyl groups was observed at around 3400  $\text{cm}^{-1}$ . This may indicate the hydrolysis of surface siloxane groups.

[0211] The foregoing discussion of the invention has been presented for purposes of illustration and description. The foregoing is not intended to limit the invention to the form or forms disclosed herein. Although the description of the invention has included description of one or more embodiments and certain variations and modifications, other variations and modifications are within the scope of the invention, e.g., as may be within the skill and knowledge of those in the art, after understanding the present disclosure. It is intended to obtain rights which include alternative embodiments to the extent permitted, including alternate, interchangeable and/or equivalent structures, functions, ranges or steps to those claimed, whether or not such alternate, interchangeable and/or equivalent structures, functions, ranges or steps are disclosed herein, and without intending to publicly dedicate any patentable subject matter.

### What is claimed is:

1. A surface modified polymer comprising:  
a bulk polymer matrix comprising a reactive functional group dispersed therein; and  
a polymeric surface which comprise a surface modifying compound that is covalently bonded to the reactive functional group.
2. The surface modified polymer of claim 1, wherein said polymer comprises a channel extending from an exterior surface of the polymer through the bulk polymer matrix, wherein the channel defines an inner surface.
3. The surface modified polymer of claim 2, wherein the inner surface comprises a surface modifying compound that is covalently bonded to the reactive functional group.
4. The surface modified polymer of claim 3, wherein said polymer comprises a plurality of layers of said bulk polymer matrix, wherein each layer of said bulk polymer matrix comprises a complementary reactive functional group dispersed therein.
5. The surface modified polymer of claim 4, wherein an interface between two bulk polymer matrix layers is covalently bonded through polymerization of the complementary reactive functional groups of said bulk polymer matrix layer.
6. The surface modified polymer of claim 1, wherein said bulk matrix comprises at least 1 reactive functional group per 10,000 monomeric units of said polymer.
7. The surface modified polymer of claim 1, wherein said polymer is an off-ratio polymer.
8. The surface modified polymer of claim 7, wherein said polymer is a silicone off-ratio polymer.
9. The surface modified polymer of claim 8, wherein said silicone off-ratio polymer is GE RTV 615.
10. The surface modified polymer of claim 9, wherein said polymer comprises at least two layers of GE RTV 615 polymers in which the reactive functional group of two adjoining layers are such that the reactive functional group of one layer is silane and the reactive functional group of the other layer is an olefin.
11. The surface modified polymer of claim 1, wherein said reactive functional group is selected from the group consisting of silane, olefin, isocyanate, hydroxyl, epoxy, and amine.
12. The surface modified polymer of claim 1, wherein said polymer is derived from polymerization of at least two different monomeric components.
13. The surface modified polymer of claim 12, wherein said reactive functional groups of a first and second monomeric components are complementary reactive functional groups of each other selected from the group consisting of an olefin, silane, hydroxyl, isocyanate, epoxy, and amine.
14. The surface modified polymer of claim 1, wherein said surface modifying compound is a biocompatible compound.
15. The surface modified polymer of claim 14, wherein said biocompatible polymer is selected from the group consisting of polyethylene glycol, poly(methyl methacrylate), poly(ethylene vinyl acetate), poly(2-hydroxyethyl methacrylate), polyvinylpyrrolidone, CH<sub>2</sub>=CH-PEG, and CH<sub>2</sub>=CH-(CH<sub>2</sub>)<sub>n</sub>-Teflon.
16. A polymeric article comprising:  
an exterior surface;  
a bulk polymer matrix comprising a reactive functional group dispersed therein; and  
a channel extending from the exterior surface through the bulk polymer matrix, wherein the channel defines an inner surface, wherein the exterior and inner surfaces comprise a surface modifying compound that is covalently bonded to the reactive functional group.
17. The polymeric article of claim 16, wherein said article is a microfluidic device.
18. The polymeric article of claim 17, wherein said channel defines a fluid flow channel of said microfluidic device.
19. The polymeric article of claim 18, wherein said surface modifying compound that is covalently attached to

said inner surface of flow channel is a stationary phase compound which is capable of separating an analyte in a fluid sample.

20. The polymeric article of claim 18, wherein said surface modifying compound that is covalently attached to said exterior surface is a biocompatible compound.

21. A method for modifying a physical property of a polymer surface comprising:

- (a) forming a bulk polymer matrix having a reactive functional group dispersed therein; and
- (b) producing a surface modified polymer by contacting a surface of the polymer with a surface modifying compound comprising a complementary functional group under conditions sufficient to form a covalent bond between the reactive functional group that is present on the polymer surface and the complementary functional group of the surface modifying compound.

22. The method of claim 21, wherein the reactive functional group is selected from the group consisting of silane, olefin, isocyanate, hydroxyl, epoxy, and amine.

23. The method of claim 21, wherein the surface modifying compound is a biocompatible compound.

24. The method of claim 23, wherein the biocompatible compound is selected from the group consisting of polyethylene glycol, poly(methyl methacrylate), poly(ethylene vinyl acetate), poly(2-hydroxyethyl methacrylate), polyvinylpyrrolidone,  $\text{CH}_2=\text{CH-PEG}$ , and  $\text{CH}_2=\text{CH-(CH}_2\text{)}_n\text{-Teflon}$ .

25. The method of claim 21, wherein the surface modifying compound render the polymer resistant to a solvent.

26. The method of claim 25, wherein the surface modifying compound is selected from the group consisting of  $\text{CH}_2=\text{CH-(CH}_2\text{)}_n\text{-Teflon}$ , and  $\text{CH}_2=\text{CH-(CH}_2\text{)}_n\text{-fluoropolymer}$ .

27. The method of claim 21, wherein said bulk polymer matrix forming step comprises admixing a first monomeric compound comprising a first reactive functional group with a second monomeric compound comprising a second reactive functional group under conditions sufficient to produce the bulk polymer matrix, wherein the first reactive functional group and the second reactive functional group are complementary functional groups.

28. The method of claim 27, wherein the first reactive functional group and the second reactive functional group are selected from the group consisting of silane, olefin, hydroxyl, isocyanate, epoxy, and amine, such that the first reactive functional group and the second reactive functional group are complementary functional groups.

29. A microfluidic device comprising:

- (a) a bulk polymer matrix comprising a reactive functional group dispersed therein;
- (b) a flow channel within said bulk polymer matrix, wherein said flow channel defines an inner surface; and
- (c) a surface modifying compound covalently attached to the reactive functional group that is present on the inner surface.

30. The microfluidic device of claim 29, wherein said device comprises a plurality of layers of said bulk polymer matrix, wherein each layer of said bulk polymer matrix comprises a reactive functional group dispersed therein.

31. The microfluidic device of claim 30, wherein two adjoining bulk polymer matrix layers are covalently bonded

to one another by the reactive functional groups that are complementary to one another.

32. The microfluidic device of claim 29, wherein said bulk polymer matrix comprises at least one reactive functional group per 1000 monomeric unit used to form said bulk polymer matrix.

33. The microfluidic device of claim 29, wherein said reactive functional group is selected from the group consisting of silane, olefin, hydroxyl, isocyanate, epoxy, and amine.

34. The microfluidic device of claim 29, wherein said bulk polymer matrix is derived from polymerization of at least two different monomeric components.

35. The microfluidic device of claim 34, wherein said reactive functional group of a first monomeric component and said reactive functional group of a second monomeric component are complementary reactive functional groups of each other selected from the group consisting of an olefin, silane, hydroxyl, isocyanate, epoxy, and amine.

36. The microfluidic device of claim 29, wherein said surface modifying compound is a biocompatible polymer.

37. The microfluidic device of claim 36, wherein said biocompatible polymer is selected from the group consisting of polyethylene glycol, poly(methyl methacrylate), poly(ethylene vinyl acetate), poly(2-hydroxyethyl methacrylate), polyvinylpyrrolidone,  $\text{CH}_2=\text{CH-PEG}$ , and  $\text{CH}_2=\text{CH-(CH}_2\text{)}_n\text{-Teflon}$ .

38. A method for producing a microfluidic device comprising a bulk polymer matrix, a flow channel within said bulk polymer matrix, wherein the flow channel defines an inner surface, and a surface modifying compound covalently attached to the inner surface, said method comprising the steps of:

- (a) producing the microfluidic device from a polymer precursor under conditions sufficient to produce the bulk polymer matrix comprising a reactive functional group dispersed within the inner surface, and
- (b) contacting the inner surface with a surface modifying compound under conditions sufficient to covalently attach the surface modifying compound to the inner surface.

39. The method of claim 38, wherein the polymer precursor comprises at least two different monomeric components.

40. The method of claim 39, wherein the reactive functional group of a first monomeric component and the reactive functional group of a second monomeric component are complementary reactive functional groups of each other selected from the group consisting of an olefin, silane, hydroxyl, isocyanate, epoxy, and amine.

41. The method of claim 38, wherein said surface modifying compound is a biocompatible polymer.

42. The method of claim 41, wherein the biocompatible polymer is selected from the group consisting of polyethylene glycol, poly(methyl methacrylate), poly(ethylene vinyl acetate), poly(2-hydroxyethyl methacrylate), polyvinylpyrrolidone,  $\text{CH}_2=\text{CH-PEG}$ , and  $\text{CH}_2=\text{CH-(CH}_2\text{)}_n\text{-Teflon}$ .

43. The method of claim 38, wherein said bulk polymer matrix comprises at least one reactive functional group per 10,000 monomeric units of said polymer.

\* \* \* \* \*